JET PROPULSION LABORATORY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

PROGRESS REPORT

Microorganism Study
JPL Contract No. 950783, under NAS 7-100

Prof. W.B. Bollen, Microbiologist
Oregon State University
Corvallis, Oregon

August 20, 1965

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Descriptive charts for 12 more isolants from the first group of 31 cultures sent to us are presented herewith. All 12 are soil diphtheroids; the only available genus for these is Corynebacterium, into which they fall readily with respect to morphology and general physiology. However, they differ from all described species, many of the biochemical reactions and cultural characters, especially pigmentation, being different. All species described in Bergey's Manual are animal pathogens or parasites, or plant pathogens. Soil diphtheroids are mentioned in the literature but none have been systematically described and named. They undoubtedly constitute a part of Winogradsky's unclassified autochthonous microflora and a new genus should be established for them. Most of the second group of cultures you submitted are also soil diphtheroids. While most of the major tests on these have been completed, photomicrographs and measurements remain to be done. When these descriptions, and descriptions of other soil diphtheroids that may be found in subsequent cultures sent to us, are completed, a complete key will be prepared and an appropriate new genus and species names will be suggested.

From the Key presented herewith, and preceeding the descriptive charts, it is apparent that the main differentiating characters are size, hydrolysis of gelatin, and reduction of nitrates. The subsequent keying characters are less significant and may be variable. It seems doubtful if the 12 isolants represent even six justifiable species. Cultures 15B2 and 15B3 differ only in color, and but slightly; on different media the colors would probably be different than on trypticase soy agar and would perhaps be even more alike, suggesting variants of a single species.

Seventeen of the third group of 48 isolants have failed to grow on transfer to TSA or in enrichment media. Of the 17, most are small cocci or short rods; five show branching.

The fourth group of isolants, from Hilgard's soils, are all growing except No. 82A. Cultural characters only have been completed.

Culture work is just starting on the recently received fifth group of isolants from the African and Chilean desert soils.

KEY TO SOIL DIPTHEROIDS

Corynebacterium. Aerobic. Gram-positive. non-spore-forming. Non-motile. Usually straight; but occasionally slightly curved, club-shaped, and irregularly stained segments. Angular and palisade formations of cells may follow characteristic snapping division. Catalase-positive. Utilize NH_4 as sole nitrogen source. Do not hydrolyze fat, nor produce indol, acetyl methyl carbinol, or hydrogen sulfide. May or may not liquefy gelatin, reduce nitrates to nitrites, or ferment sugars. If sugars fermented, only slight amount of acid produced.

I. Large size. 2.5-3.6 x 0.8-0.9μ.
Casein, gelatin, and starch not hydrolyzed. Urea hydrolyzed.
Ammonia from peptone.

A. Nitrates reduced. Methylene blue reduced.

1. Salt tolerance 2%..... 15A1
2. Salt tolerance 10%.

a. Dull..... 19X
b. Glistening.

(1). Color Lt. Mellon Yellow.... 15A
(2). Color Lt. Apricot..... 19G1

B. Nitrates not reduced.

1. Methylene blue reduced.

a. Gelatin liquefaction..... 122B
b. No gelatin liquefaction..... 19Y

2. Methylene blue not reduced..... 19E1

II. Small size. 0.9-1.1 x 0.4-0.6u.

A. Gelatin hydrolyzed..... 16
B. Gelatin not hydrolyzed.

1. Nitrate reduced. Starch positive... 13C

2. Nitrate not reduced. Starch, casein, and urea not hydrolyzed. Methylene blue not reduced.

a. Ammonia from peptone..... 14D
b. No ammonia from peptone.

(1). Color Flame..... 15B3
(2). Color Cherry..... 15B2

III. Systematic study of isolants 14E, 19E2, and 20F has not been completed because the cultures have failed to continue to grow on transfer. Sugar utilization, colony descriptions, photography, and measurements remain unfinished. Characters so far determined indicate these isolants are soil diphtheroids; 14E falls into the small size group, 19E2 and 20F are in the large group.

Name of organism Corynebacterium sp.* Studied by Dr. W. B. Bollen Culture No. 130

Source White Mountain Habitat Soil Date August 2, 1965

Descriptions (*Underscore required terms.*) | Sketches

CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 28c.

Vegetative cells: Age: 24hr

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: - Flagella: No flagella

Size: 1.10 x 0.50 Irregular forms:
1.0 - 1.3 x 0.5

Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

SEE PAGE 3 FOR PHOTOMICROGRAPHS

STAINING CHARACTERISTICS

Gram: + Age: 24hr Method: Kopeloff's (modified)

Special stains:

AGAR STROKE Age: 24 hr. Temp. 28°c.

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: ; *fluorescent, iridescent, photogenic.*

5ga (Peach)

moderate to abundant after 48hr.

AGAR COLONIES Age: 8 days Temp. 28°c.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.* rugose

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*

NUTRIENT BROTH Age: 8 days Temp. 28°c.

Surface growth: *none, ring, pellicle, flocculent; membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 12 day Temp. 25°c.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant	61a	Brite Coral Red	
Soybean Infusion agar	4ea	Light Apricot	abundant
Fat agar	4ea	Light Apricot	abundant
Glucose nitrate	4ca	Flesh Pink	abundant

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth Carbohydrate: 1% Indicator: BCP	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	+	+	+	-	
Acid in days					
Gas in 2 days	+	+	+	-	
Gas in days					

ACTION ON MILK Temp. 28 °C.

Indicator: Litmus	Days 35					
Reaction	=					
Acid curd	=					
Rennet curd	=					
Peptonization	=					
Reduction (before coagulation)	=					

* Culturally similar to C. equi, an animal pathogen.

2

ACTION ON NITRATES

Medium: 1% KNO_3 broth Temp. 28°C.

Nitrite: +3 d. ; ..... d. ; ..... d.

Gas (N): -3 d. ; ..... d. ; ..... d.

INDOLE PRODUCTION

Medium: Tryptophan broth Age: 10 days

Method: Kovac's Temp. 28C.

Indole: *present, absent.*

HYDROGEN SULFIDE PRODUCTION

Medium: Lead Acetate & Thio iron Age: 10 day Temp. 28C.

H_2S: *present, absent.*

TEMPERATURE RELATIONS

Growth in refrigerator (10°C.): *present, absent.*

Growth at room temperature (28°C.): *present, absent.*

Growth at 37° C.: *present, absent.*

Growth at 50° C.: *present, absent.*

RELATION TO FREE OXYGEN Catalase -Positive

Medium: TSA & Dextrose Age: 10 day

Method: Shake tubes Temp. 28C.

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

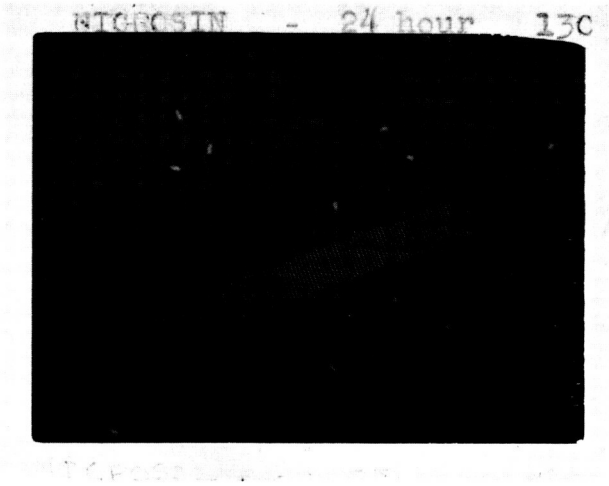
Anaerobic growth: *present, absent.*

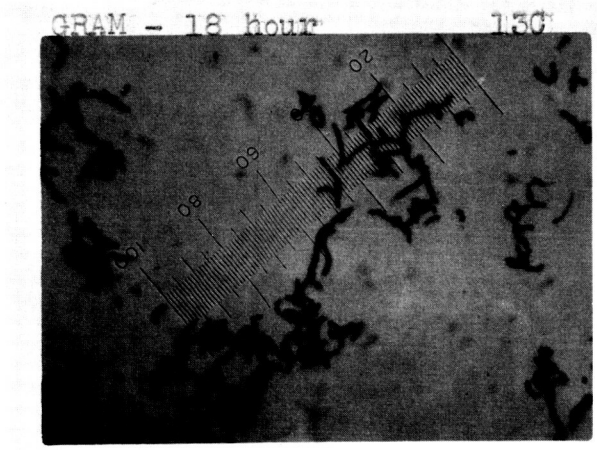
Pasteurization survival, 80°C. 10 minutes: Negative

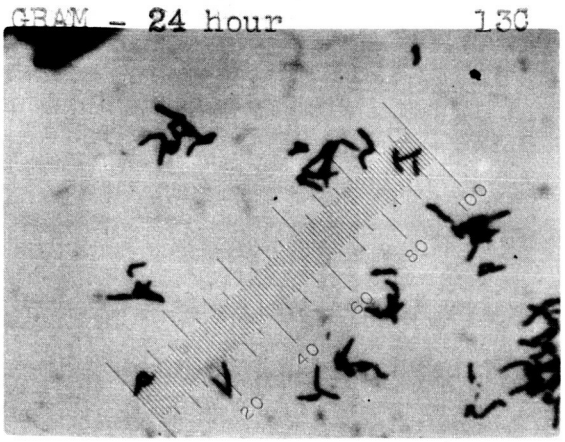
ADDITIONAL TESTS

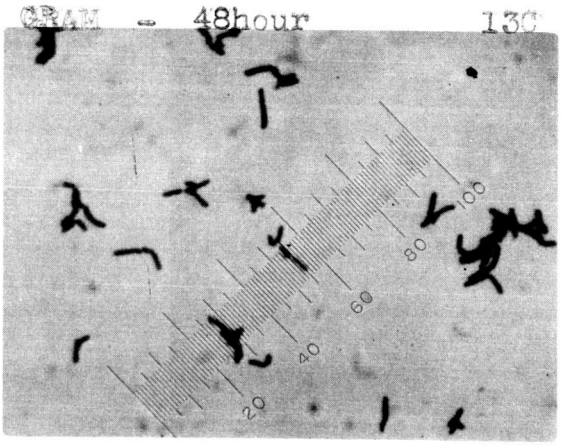
Casein Hydrolysis:		Negative
Fat Hydrolysis:		Negative
Gelatin Hydrolysis:		Negative
Starch Hydrolysis:		Positive
Urea Hydrolysis:		Negative
NH_4 from Peptone:		Positive
Metabolism:		Nonoxidizer-nonfermentor
Acetyl methyl carbinol:		Negative
NH_4 as sole Nitrogen source:		Positive
Sole Carbon sources:	Citrate -	Positive
	Glucose -	Positive
	Sucrose -	Positive
	Xylose -	Positive - slight
Methylene blue reduction:		Positive
Salt tolerances:	2% -	Positive
	7% -	Negative
	10% -	Negative
Cellulose Digestion:		Negative
Selenite-nutrient agar:		Positive - slight

1000x


NIGROSIN - 24 hour 13C


GRAM - 18 hour 13C


GRAM - 24 hour 13C


GRAM - 48hour 13C

Cultural Characteristics of Bacterial Colonies

Culture No. 13C

I. Surface Colonies. Age 8 days, on TSA medium.

a. Microscopic appearance.

1. Size, 3mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.

Topography- smooth, rough, wrinkled, contoured, straited, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 5ga (Peach)

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, filamentous, curled.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.,

d. Odor. Special

Name of organism Corynebacterium sp.* Studied by Dr. W.B. Bollen Culture No. 14D

Source White Mountain Habitat Soil Date August 2, 1965

Descriptions (*Underscore required terms.*)	Sketches
CELL MORPHOLOGY Medium: Trypticase soy agar Temp 28 °C. Vegetative cells: Age: 24 hr. Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.* Motility in broth: Flagella: No flagella Size: 0.9-1.5 x 0.5-1.0 μ Irregular forms: coccoid Sporangia: *none, rods, spindles, clavate, drumstick.* Age: Endospores: Shape: *spherical, ellipsoid, cylindrical.* Position: *central to excentric, terminal, subterminal.*	SEE PHOTOMICROGRAPHS PAGE 7
STAINING CHARACTERISTICS Gram: + Age: 24 hr Method: Kopeloff's (modified) Special stains:	
AGAR STROKE Age: 24 hr. Temp 28 °C. Amount of growth: *scanty, moderate, abundant.* Form: *filiform, echinulate, beaded, spreading, rhizoid.* Consistency: *butyrous, viscid, membranous, brittle.* Chromogenesis: 6pa (Brite Coral Red) ; *fluorescent, iridescent, photogenic.* Non-water soluble pigment	
AGAR COLONIES Age: 8 days Temp. 28°C. Form: *punctiform, circular, filamentous, rhizoid, irregular.* Elevation: *effuse, flat, raised, convex.* Surface: *smooth, contoured, radiate, concentric, rugose.* Margin: *entire, undulate, erose, filamentous, curled.* Density: *opaque, translucent.*	
NUTRIENT BROTH Age: 12 day Temp. 25°C. Surface growth: *none, ring, pellicle, flocculent, membranous.* Subsurface growth: *none, turbid, granular.* Amount of growth: *scanty, moderate, abundant.* Sediment: *none, granular, flocculent, viscid, flaky.*	
GELATIN STAB Age: 12 day Temp. 25°C. Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.* Rate: *slow, moderate, rapid.*	

OTHER MEDIA Age: Temp. °C.

Potato slant -			No Growth
Soybean Inf. agar -	4na	Brite Orange	Moderate
Glucose nitrate agar-	41a	Orange	Abundant
Fat agar -	4pa	Brite Orange	Abundant

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth
Carbohydrate: 1%
Indicator: BCP

	Glucose	Lactose	Sucrose	Xylose	
Acid in 10 days	+	+	+	-	
Acid in days					
Gas in 10 days	+	+	+	+	
Gas in days					

ACTION ON MILK Temp. 25 °C.

Indicator:	Days						
Litmus	35						
Reaction	+						
Acid curd	+						
Rennet curd	-						
Peptonization	+						
Reduction (before coagulation)	-						

* No agreement with any described species in Bergey's Manual.

6

ACTION ON NITRATES

Medium: 1% KNO_3 broth Temp. 28°C.

Nitrite: .....d. ; .....d. ; -3.....d.

Gas (N): .....d. ; .....d. ; -3.....d.

INDOLE PRODUCTION

Medium Tryptophane broth Age: 10 day

Method: KOVAC'S Temp. 28 °C.

Indole: *present, absent.*

HYDROGEN SULFIDE PRODUCTION

Medium Pb Acetate & Thio Iron Age: 10 da. Temp. 28°C.

H_2S: *present, absent.*

TEMPERATURE RELATIONS

Growth in refrigerator (4 °C.): *present, absent.*

Growth at room temperature (10 °C.): *present, absent.*

Growth at 37° C.: *present, absent.*

Growth at 45° C.: *present, absent.*

RELATION TO FREE OXYGEN Catalase: Positive

Medium Dextrose-Nutrient Agar Age: 10 days

Method: Shake tubes Temp. 25 °C.

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

Anaerobic growth: *present, absent.*

ADDITIONAL TESTS

Pasteurization survival, 80°C. 10 minutes: Negative

Casein Hydrolysis: Negative

Fat Hydrolysis: Negative

Gelatin Hydrolysis: Negative

Starch Hydrolysis: Negative

Urea Hydrolysis: Negative

NH_4 from Peptone: Positive

Metabolism: Nonoxidative-nonfermentative

Acetyl methyl carbinol: Negative

NH_4 as sole Nitrogen source: Positive

Sole Carbon sources:

Citrate -	Positive - slight
Glucose -	Positive
Sucrose -	Positive - slight
Xylose -	Positive - slight

Methylene blue reduction: Negative

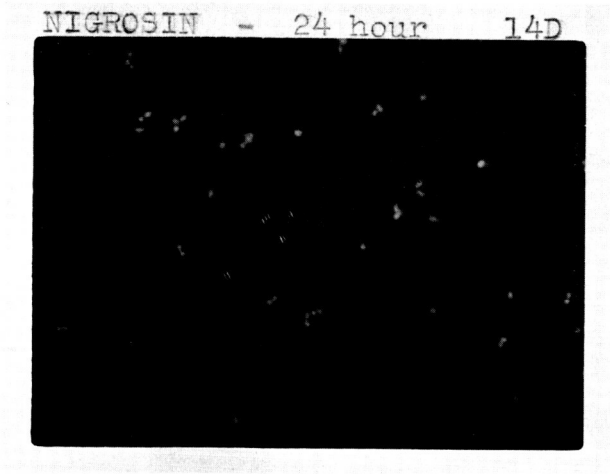
Salt tolerances:

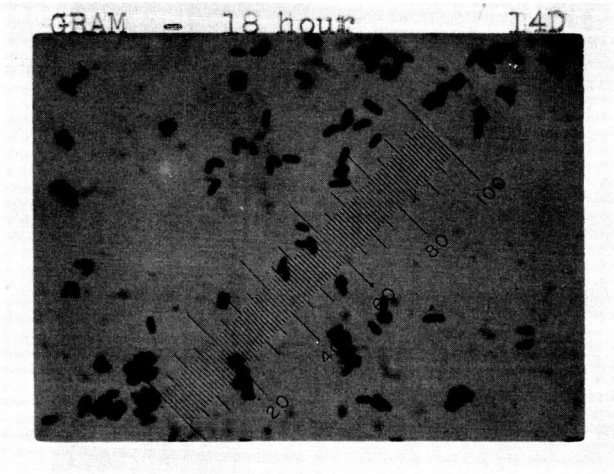
2% -	Positive
7% -	Positive
10% -	Positive

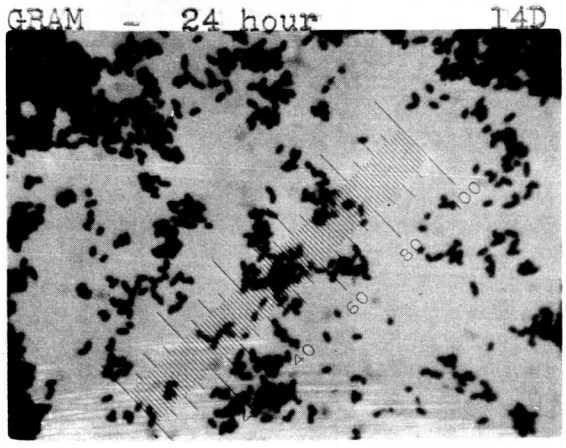
Cellulose Digestion: Negative

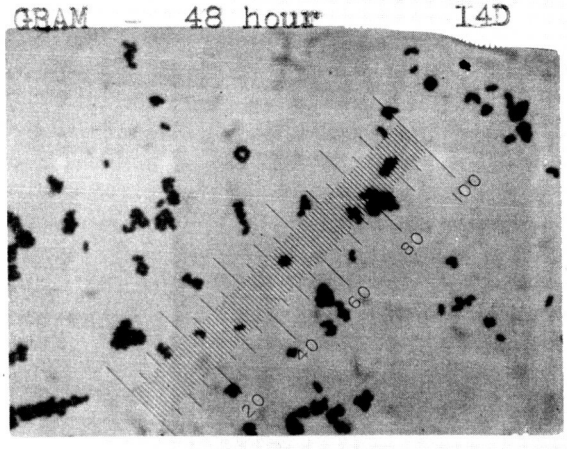
Selenite-nutrient agar: Positive

1000x


NIGROSIN - 24 hour 14D


GRAM - 18 hour 14D


GRAM - 24 hour 14D


GRAM - 48 hour 14D

Cultural Characteristics of Bacterial Colonies

Culture No. 14D

I. Surface Colonies. Age 8 days, on TSA medium.

a. Microscopic appearance.

1. Size 2mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.

Topography- smooth, rough, wrinkled, contoured, straited, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 6 pa (Brite Coral Red)

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, filamentous, curled.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.

d. Odor. cooking cauliflower

Name of organism Corynebacterium sp.* Studied by Dr. W.B. Bollen Culture No. 15A

Source White Mountain Habitat Soil Date August 2, 1965

Descriptions (*Underscore required terms.*)	Sketches

CELL MORPHOLOGY Medium: Trypticase Soy Agar Temp. 25 °C.

Vegetative cells: Age:

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.*

Motility in broth: - Flagella: -

Size: 3.4 x .9 1.0 - 5.0 x .75 - 1.0 μ Irregular forms:

Sporangia: *none, rods, spindles, ellipsoid, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

SEE PAGE 11 FOR PHOTOMICROGRAPHS

STAINING CHARACTERISTICS

Gram: + Age: 24hr. Method: Kopeloff (modified)

Special stains:

AGAR STROKE Age: 24 hr. Temp. 25°C.

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: *; fluorescent, iridescent, photogenic.*

3ea Lt. Mellon Yellow

AGAR COLONIES Age: 8 day Temp. 25°C.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.*

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*

NUTRIENT BROTH Age: 2 day Temp. 25°C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

GELATIN STAB Age: 5 day Temp 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant -	4ga	Nude Tan	Abundant
Soybean Infusion agar -		White	Moderate
Glucose nitrate agar -	3ca	Pearl Pink	Abundant
Fat agar -		Clear to White	Scant

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth Carbohydrate: 1% Indicator: BCP

	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	+	=	=	=	
Acid in 10 days	=	=	=	=	
Gas in 2 days	=	=	=	=	
Gas in 10 days	=	=	=	=	

ACTION ON MILK Temp. 25 °C.

Indicator: Litmus	Days						
	8						
Reaction	ALKALINE						
Acid curd							
Rennet curd							
Peptonization							
Reduction (before coagulation)							

* Soil Actinomycete; no described species.

Resembles closely 15A1.

ACTION ON NITRATES
Medium: 1%KNO3 broth Temp. 28c.
Nitrite: .....d. ; .....d. ; + 3 d.
Gas (N): .....d. ; .....d. ; - 3 d.

INDOLE PRODUCTION
Medium: Tryptophane broth Age: 10 day
Method: Kovac's Temp. 28c.
Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION
Medium: Pb Acetate & Thio-iron Age: 10 day Temp. 25c.
H_2S: *present*, *absent*.

TEMPERATURE RELATIONS
Growth in refrigerator (10°C.): *present*, *absent*.
Growth at room temperature (28°C.): *present*, *absent*.
Growth at 37° C.: *present*, *absent*.
Growth at 50° C.: *present*, *absent*.

RELATION TO FREE OXYGEN- Catalase: Positive
Medium Dextrose-Nutrient Agar 10day
Method: Shake Tubes Temp. 28c.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

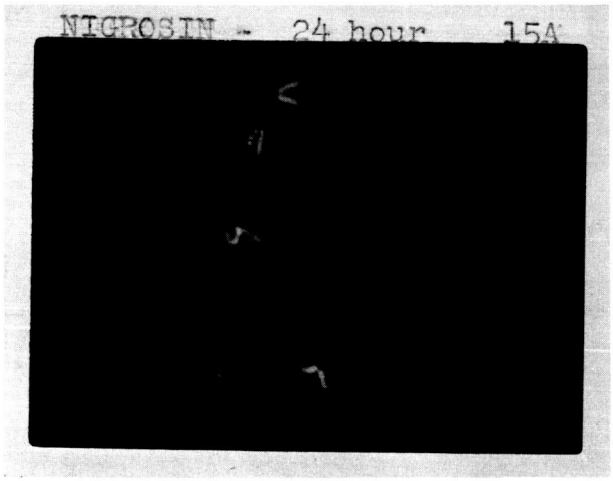
Anaerobic growth: *present*, *absent*.

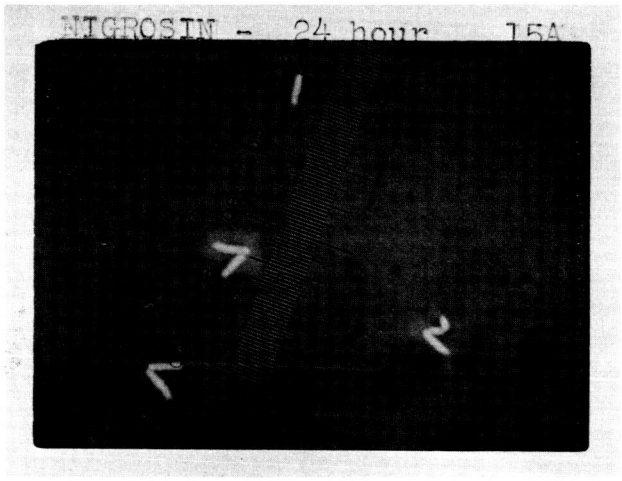
Pasteurization survival, 80°C. 10 minutes: Negative

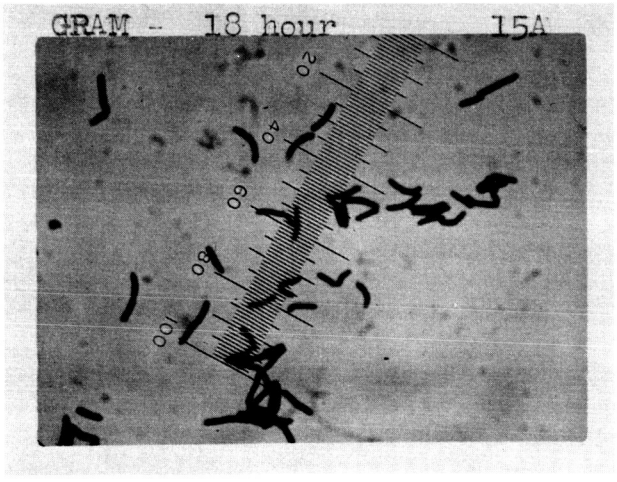
ADDITIONAL TESTS

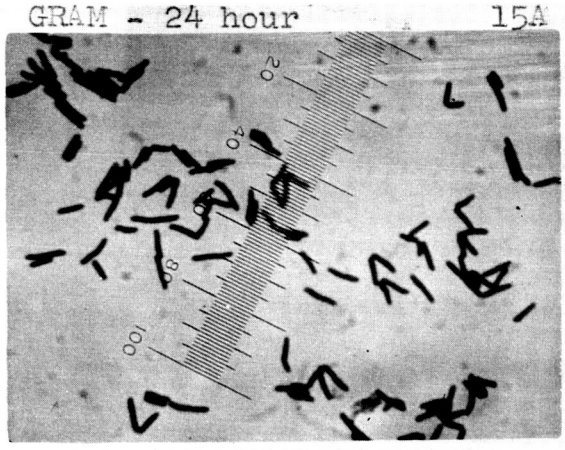
Casein Hydrolysis:	Negative
Fat Hydrolysis:	Negative
Gelatin Hydrolysis:	Negative
Starch Hydrolysis:	Negative
Urea Hydrolysis:	Positive
NH_4 from Peptone:	Positive
Metabolism:	Oxidizes Glucose, Sucrose, Xylose Nonox-nonferm. Lactose
Acetyl methyl carbinol:	Negative
NH_4 as sole Nitrogen source:	Positive
Sole Carbon sources: Citrate -	Positive
Glucose -	Positive
Sucrose -	Positive
Xylose -	Positive - slight
Methylene blue reduction:	Positive
Salt tolerances: 2% -	Positive
7% -	Positive
10% -	Positive
Cellulose Digestion:	Negative
Selenite-nutrient agar:	Positive

1000x


NIGROSIN - 24 hour 15A


NIGROSIN - 24 hour 15A


GRAM - 18 hour 15A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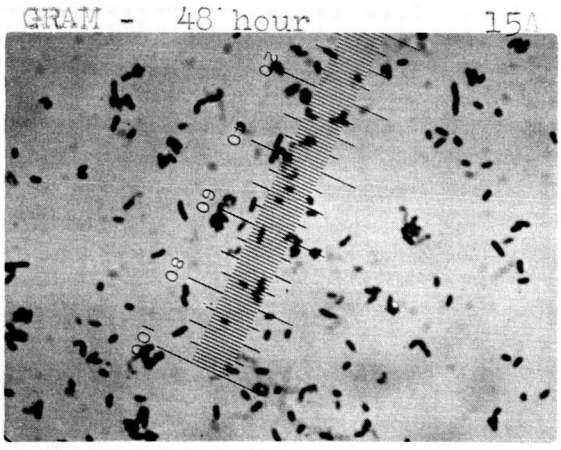

GRAM - 48 hour 15A

Cultural Characteristics of Bacterial Colonies

Culture No. 15A

I. Surface Colonies. Age 8 days, on TSA medium.

a. Microscopic appearance.

1. Size, 4mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.

Topography- smooth, rough, wrinkled, contoured, straited, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 3ea St. Mellon Yellow

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, filamentous, curled.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.,

d. Odor. Fecal

Name of organism Corynebacterium sp.* Studied by Dr. W. B. Bollen Culture No. 15A1

Source White Mountain Habitat Soil Date August 2, 1965

Descriptions (*Underscore required terms.*)	Sketches
CELL MORPHOLOGY Medium Trypticase soy agar Temp. 25°C. Vegetative cells: Age 24 hr. Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.* Motility in broth: — Flagella: — Size: 3.45 x 0.79 μ Irregular forms: 1.5-10.0 x 0.5-1.0 μ Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age: Endospores: Shape: *spherical, ellipsoid, cylindrical.* Position: *central to excentric, terminal, subterminal.*	SEE PAGE 15 FOR PHOTOMICROGRAPHS
STAINING CHARACTERISTICS Gram: + Age 24 hr Method: Kopeloff (modified) Special stains:	
AGAR STROKE Age: 24 hr. Temp. 25°C. Amount of growth: *scanty, moderate, abundant.* Form: *filiform, echinulate, beaded, spreading, rhizoid.* Consistency: *butyrous, viscid, membranous, brittle.* Chromogenesis: ; *fluorescent, iridescent, photogenic.* 3ea Lt. Mellon Yellow	
AGAR COLONIES Age: 3 day Temp. 25°C. Form: *punctiform, circular, filamentous, rhizoid, irregular.* Elevation: *effuse, flat, raised, convex.* Surface: *smooth, contoured, radiate, concentric, rugose.* Margin: *entire, undulate, erose, filamentous, curled.* Density: *opaque, translucent.*	
NUTRIENT BROTH Age: 2 day Temp. 25°C. Surface growth: *none, ring, pellicle, flocculent, membranous.* Subsurface growth: *none, turbid, granular.* Amount of growth: *scanty, moderate, abundant.* Sediment: *none, granular, flocculent, viscid, flaky.*	
GELATIN STAB Age: 7 day Temp. 25°C. Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.* Rate: *slow, moderate, rapid.*	

OTHER MEDIA Age: Temp. °C.

Potato slant:	4gc	Nude Tan	Abundant
Soybean Infusion agar:	4ig	Fawn	Abundant
Glucose nitrate agar:		White	Scant
Fat agar:	3oa	Pearl Pink	Abundant

FERMENTATION Temp. 25 °C.

Medium Nutrient broth
Carbohydrate: 1 %
Indicator BCP

	Glucose	Lactose	Sucrose	Xylose	
Acid in 10 days	-	-	-	-	
Acid in days					
Gas in 10 days					
Gas in days					

ACTION ON MILK Temp. 25 °C.

Indicator:	Days					
Litmus	4					
Reaction	ALKALINE					
Acid curd						
Rennet curd						
Peptonization						
Reduction (before coagulation)						

* Soil diptheroid; no described species.
Resembles closely 15A.

ACTION ON NITRATES

Medium: 1% KNO_3 Broth Temp 25 °C.

Nitrite: .....d. ; .....d. ; +3 d.

Gas (N): .....d. ; .....d. ; -3 d.

INDOLE PRODUCTION

Medium: Tryptophane broth Age: 10 day

Method: Kovac's Temp. 28°C.

Indole: *present, absent.*

HYDROGEN SULFIDE PRODUCTION

Medium: Pb Acetate & Thio-iron Age: 10 day Temp 28 °C.

H_2S: *present, absent.*

TEMPERATURE RELATIONS

Growth in refrigerator (10 °C.): *present, absent.*

Growth at room temperature (28 °C.): *present, absent.*

Growth at 37° C.: *present, absent.*

Growth at 50° C.: *present, absent.*

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose-nutrient agar 10 day

Method: Shake tubes Temp. 28°C.

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

Anaerobic growth: *present, absent.*

Uniform growth throughout

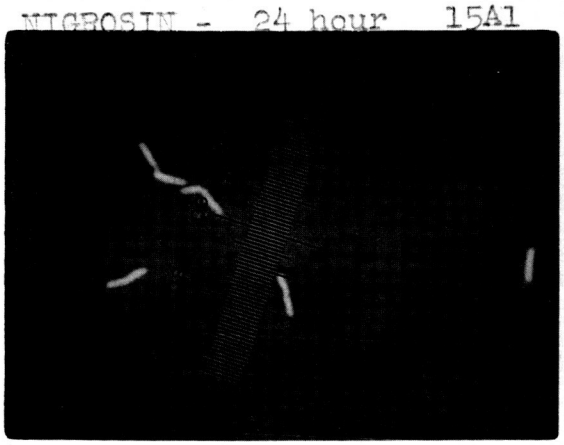
Pasteurization survival, 80°C. 10 minutes: Negative

ADDITIONAL TESTS

Casein Hydrolysis:	Negative
Fat Hydrolysis:	Negative
Gelatin Hydrolysis:	Negative
Starch Hydrolysis:	Negative
Urea Hydrolysis:	Positive
NH_4 from Peptone:	Positive
Metabolism:	Ferments Sucrose Oxidizes Glucose Nonox.-nonferm. Lactose & Xylose
Acetyl methyl carbinol:	Negative
NH_4 as sole Nitrogen source:	Positive
Sole Carbon sources: Citrate -	Positive
Glucose -	Positive
Sucrose -	Positive
Xylose -	Positive - slight
Methylene blue Reduction:	Positive
Salt tolerances: 2% -	Positive
7% -	Negative
10% -	Negative
Cellulose Digestion:	Negative
Selenite-nutrient agar:	Positive

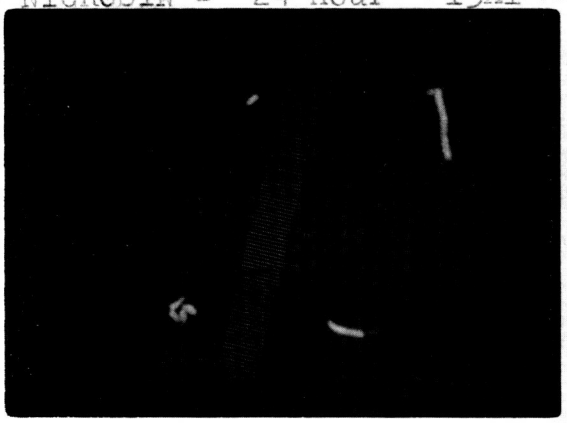
1000x

NIGROSIN - 24 hour 15A1

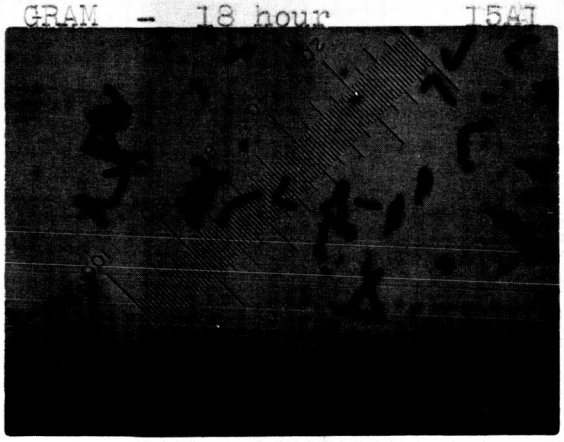


NIGROSIN - 24 hour 15A1

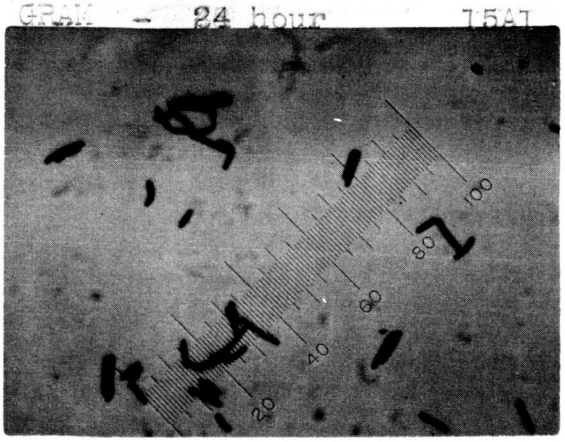
NIGROSIN - 24 hour 15A1



GRAM - 18 hour 15A1



GRAM - 24 hour 15A1



GRAM - 48 hour 15A1

Cultural Characteristics of Bacterial Colonies

Culture No. 15A1

I. Surface Colonies. Age 8 days, on TSA medium.

a. Microscopic appearance.

1. Size, 3mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.

Topography- smooth, rough, wrinkled, contoured, straited, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 3ea St. Mellon yellow

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, filamentous, curled.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.,

d. Odor. cooking cabbage

Name of organism Corynebacterium sp.* Studied by Dr. W.B.Bollen Culture No 15B2

Source White Mountain Habitat Soil Date August 5, 1965

Descriptions (*Underscore required terms.*)	Sketches
CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 25 °C. Vegetative cells: Age: 24 hour. Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.* Motility in broth: – Flagella: – Size: 0.90 x 0.6µ Irregular forms: coccoid 0.5-1.5 x 0.5-2.0 µ Sporangia: *none, rods, spindles, ellipsoid, clavate, drumstick.* Age: Endospores: Shape: *spherical, ellipsoid, cylindrical.* Position: *central to excentric, terminal, subterminal.*	SEE PAGE 19 FOR PHOTOMICROGRAPHS
STAINING CHARACTERISTICS Gram: + Age: 24hr Method: Kopeloff (modified) Special stains:	
AGAR STROKE Age: 24 hr. Temp. 25°C. Amount of growth: *scanty, moderate, abundant.* Form: *filiform, echinulate, beaded, spreading, rhizoid.* Consistency: *butyrous, viscid, membranous, brittle.* Chromogenesis: ; *fluorescent, iridescent, photogenic.* 7nc Cherry	
AGAR COLONIES Age: 8 day Temp 25 °C. Form: *punctiform, circular, filamentous, rhizoid, irregular.* Elevation: *effuse, flat, raised, convex.* Surface: *smooth, contoured, radiate, concentric, rugose.* Margin: *entire, undulate, erose, filamentous, curled.* Density: *opaque, translucent.*	
NUTRIENT BROTH Age: 2 day Temp. 25°C. Surface growth: *none, ring, pellicle, flocculent, membranous.* Subsurface growth: *none, turbid, granular.* Amount of growth: *scanty, moderate, abundant.* Sediment: *none, granular, flocculent, viscid, flaky.*	
GELATIN STAB Age: 12 day Temp 25 °C. Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.* Rate: *slow, moderate, rapid.*	
OTHER MEDIA Age: Temp. °C. Potato slant: No Growth Soybean Infusion agar: Scant Fat agar: 61a Lt Coral Red abundant Glucose nitrate agar: No Growth	

FERMENTATION	Temp. 25 °C.				
Medium Nutrient broth Carbohydrate: 1 % Indicator: BCP	Glucose	Lactose	Sucrose	Xylose	
Acid in 10 days	=	=	=	=	
Acid in days					
Gas in 10 days	=	=	=	=	
Gas in days					

ACTION ON MILK	Temp. 25 °C.					
Indicator:	Days					
Litmus	21					
Reaction	VERY SLIGHT					
Acid curd	ALKALINE					
Rennet curd						
Peptonization						
Reduction (before coagulation)						

* Soil diptheroid; no described species. Resembles 15B3 closely.

18

ACTION ON NITRATES
Medium: 1% KNO_3 broth Temp. 28c.
Nitrite: .....d. ; .....d. ; -3 d.
Gas (N): .....d. ; .....d. ; -3 d.

INDOLE PRODUCTION
Medium: Tryptophane broth Age: 10 day
Method: Kovac's Temp. 28°C.
Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION
Medium: Pb Acetate & Thio-iron Age: 10 day
H_2S: *present*, *absent*. Temp. 28c.

RELATION TO FREE OXYGEN- Catalase: Positive
Medium: Dextrose-Nutrient agar 10 day
Method: Shake tubes Temp. 28°C.

TEMPERATURE RELATIONS
Growth in refrigerator (10°C.): *present*, *absent*.
Growth at room temperature (28C.): *present*, *absent*.
Growth at 37° C.: *present*, *absent*.
Growth at 50° C.: *present*, *absent*.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

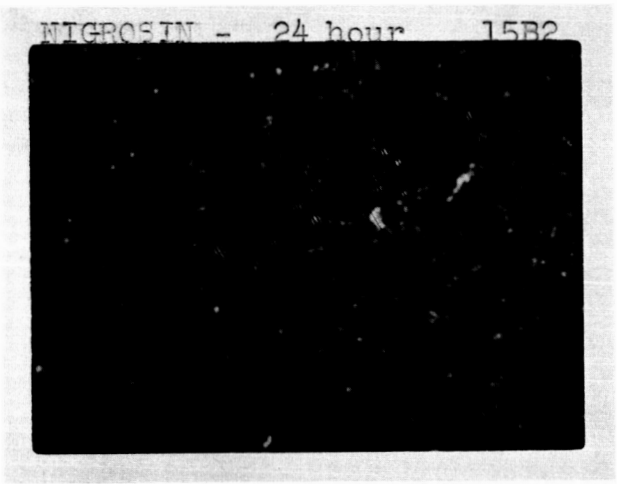
Anaerobic growth: *present*, *absent*.

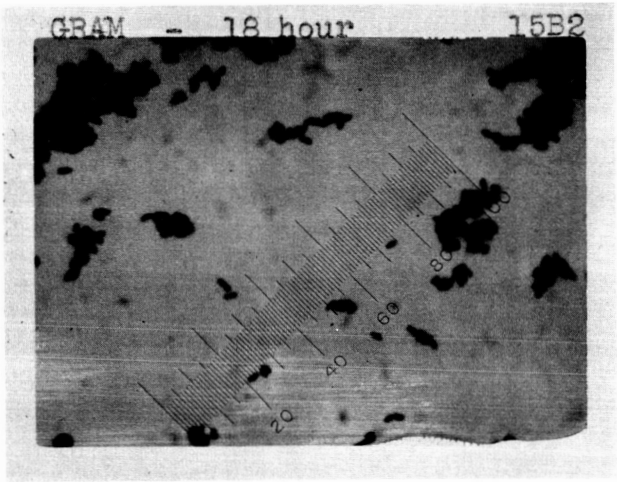
Pasteurization survival, 80°C. 10 minutes: Negative

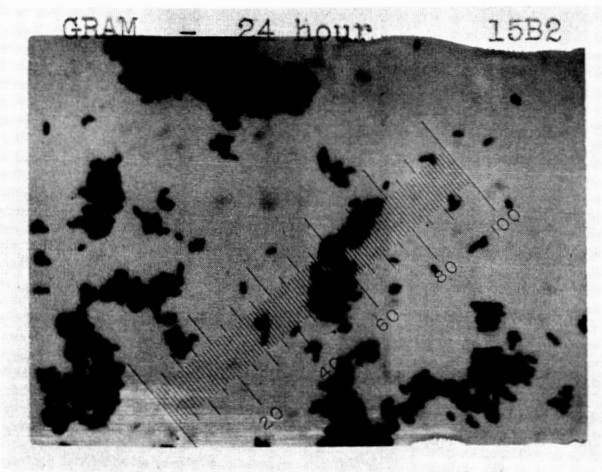
ADDITIONAL TESTS

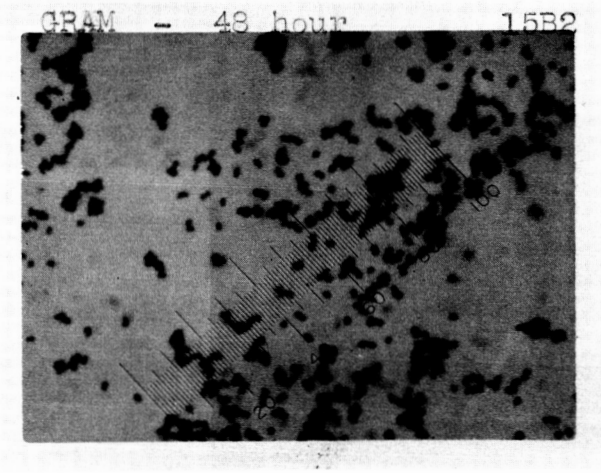
Casein Hydrolysis:	Negative
Fat Hydrolysis:	Negative
Gelatin Hydrolysis:	Negative
Starch Hydrolysis:	Negative
Urea Hydrolysis:	Negative
NH_4 from Peptone:	Negative
Metabolism:	Nonox.-nonferm.
Acetyl methyl carbinol:	Negative
NH_4 as sole Nitrogen source:	Positive
Sole Carbon sources: Citrate -	Positive - slight
Glucose -	Positive
Sucrose -	Positive
Xylose -	Positive - slight
Methylene blue Reduction:	Negative
Salt tolerances: 2% -	Positive
7% -	Positive
10% -	Negative
Cellulose Digestion:	Negative
Selenite-nutrient agar:	Positive

1000x


NIGROSIN - 24 hour 15B2


GRAM - 18 hour 15B2


GRAM - 24 hour 15B2


GRAM - 48 hour 15B2

Cultural Characteristics of Bacterial Colonies

Culture No. 1582

I. Surface Colonies. Age 8 days, on TSA medium.

a. Microscopic appearance.

1. Size, 1 mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.

Topography- smooth, rough, wrinkled, contoured, straited, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 7nc Cherry

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, filamentous, curled.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.,

d. Odor. cooking cauliflower

Name of organism Corynebacterium sp.* Studied by Dr. W.B.Bollen Culture No. 15B3

Source White Mountain Habitat Soil Date August 5, 1965

Descriptions (*Underscore required terms.*)	Sketches
CELL MORPHOLOGY Medium Trypticase soy agar Temp. 25 °C. Vegetative cells: Age: Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.* Motility in broth: Flagella: Size: 0.5-1.5 x 0.5-0.8µ Irregular forms: coccoid Sporangia: *none, rods, spindles, ellipsoids, clavate, drumstick.* Age: Endospores: Shape: *spherical, ellipsoid, cylindrical.* Position: *central to eccentric, terminal, subterminal.*	SEE PAGE 23 FOR PHOTOMICROGRAPHS
STAINING CHARACTERISTICS Gram: + Age: 24hr. Method Kopeloff (modified) Special stains:	
AGAR STROKE Age: 18 hour Temp. 25 °C. Amount of growth: *scanty, moderate, abundant.* Form: *filiform, echinulate, beaded, spreading, rhizoid.* Consistency: *butyrous, viscid, membranous, brittle.* Chromogenesis: ; *fluorescent, iridescent, photogenic.* Gm+ na Flame	
AGAR COLONIES Age: 8 day Temp. 25 °C. Form: *punctiform, circular, filamentous, rhizoid, irregular.* Elevation: *effuse, flat, raised, convex.* Surface: *smooth, contoured, radiate, concentric, rugose.* Margin: *entire, undulate, erose, filamentous, curled.* Density: *opaque, translucent.*	
NUTRIENT BROTH Age: 3 day Temp. 25 °C. Surface growth: *none, ring, pellicle, flocculent, membranous.* Subsurface growth: *none, turbid, granular.* Amount of growth: *scanty, moderate, abundant.* Sediment: *none, granular, flocculent, viscid, flaky.*	
GELATIN STAB Age: 30 day Temp. 25 °C. Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.* Rate: *slow, moderate, rapid.*	
OTHER MEDIA Age: Temp. °C. Potato slant: NO Growth Soybean Infusion agar: Scant Fat agar: 5pa Brite Orange Abundant Glucose nitrate agar: Scant	

FERMENTATION Temp. 25 °C.	Glucose	Lactose	Sucrose	Inositol	
Medium: Nutrient broth Carbohydrate: 1 % Indicator: BCP					
Acid in 10 days	=	=	=	-	
Acid in days					
Gas in 10 days	=	=	=	=	
Gas in days					

ACTION ON MILK Temp. 25 °C.	Days					
Indicator: Litmus	21					
Reaction	VERY	SLIGHTLY				
Acid curd		ALKALINE				
Rennet curd						
Peptonization						
Reduction (before coagulation)						

* Soil diptheroid; no described species.
Resembles 15B2 closely.

22

ACTION ON NITRATES

Medium: 1% KNO_3 broth Temp 28 °C.

Nitrite: .....d. ; .....d. ; -3.....d.

Gas (N): .....d. ; .....d. ; -3.....d.

INDOLE PRODUCTION

Medium: Tryptophane broth Age: 10 day

Method: Kovac's Temp. 28 °C.

Indole: *present, absent.*

HYDROGEN SULFIDE PRODUCTION

Medium. Pb Acetate & Thio-iron Age: 10 da. Temp. 28c.

H_2S: *present, absent.*

RELATION TO FREE OXYGEN - Catalase: Positive

Medium: Dextrose-nutrient agar Age:

Method: Shake tubes Temp. 28 C.

TEMPERATURE RELATIONS

Growth in refrigerator (10 °C.): *present, absent.*

Growth at room temperature (28C.): *present, absent.*

Growth at 37° C.: *present, absent.*

Growth at 50° C.: *present, absent.*

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

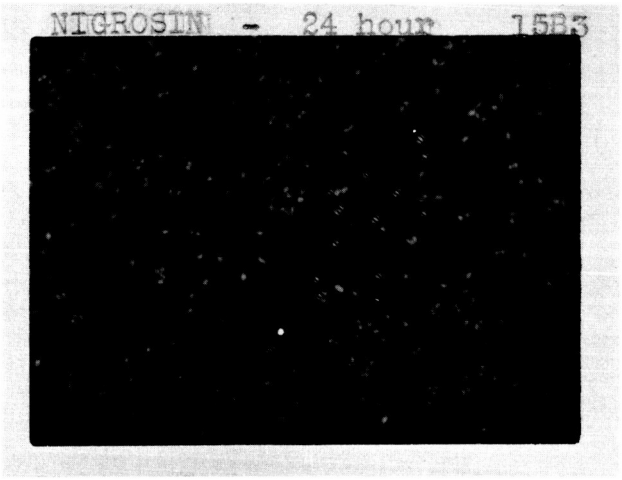
Anaerobic growth: *present, absent.*

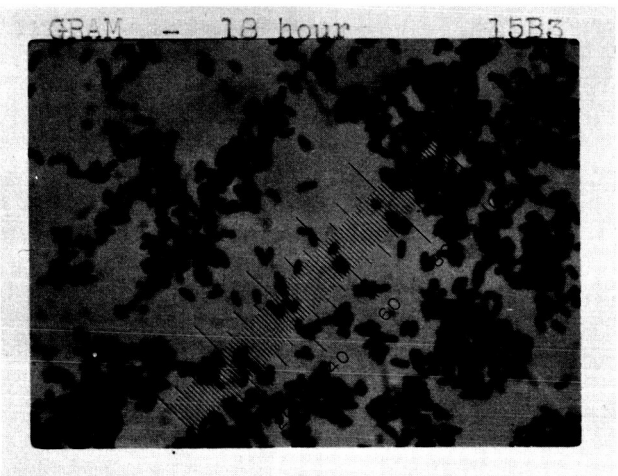
Pasteurization survival, 80°C. 10 minutes: Negative

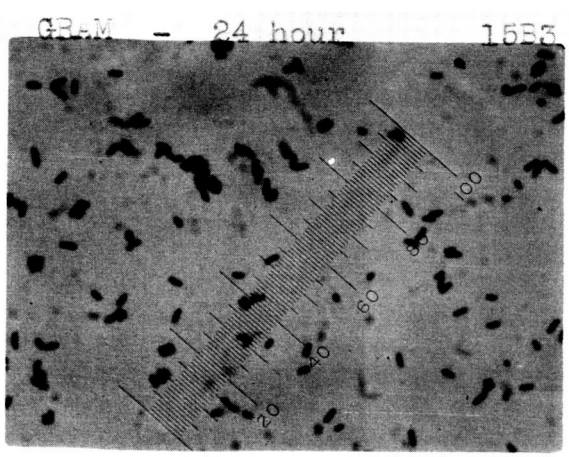
ADDITIONAL TESTS

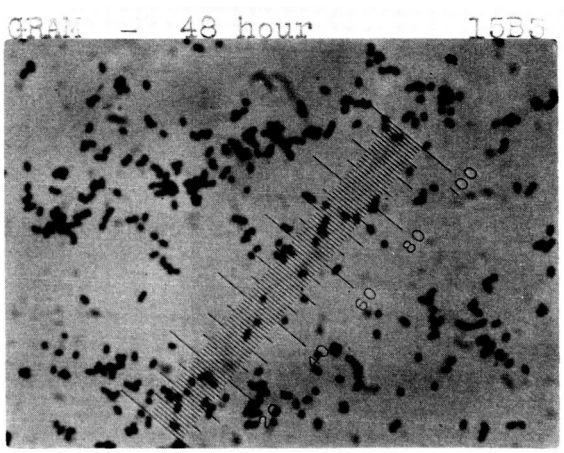
Casein Hydrolysis:	Negative
Fat Hydrolysis:	Negative
Gelatin Hydrolysis:	Negative
Starch Hydrolysis:	Negative
Urea Hydrolysis:	Negative
NH_4 from Peptone:	Negative
Metabolism:	Nonox.-nonferm.
Acetyl methyl carbinol:	Negative
NH_4 as sole Nitrogen source	Positive
Sole Carbon sources: Citrate -	Positive - slight
Glucose -	Positive
Sucrose -	Positive
Xylose -	Positive - slight
Methylene blue Reduction:	Negative
Salt tolerances: 2% -	Positive
7% -	Positive
10% -	Positive
Cellulose Digestion:	Negative
Selenite nutrient agar:	Positive

1000x


NIGROSIN - 24 hour 15B3


GRAM - 18 hour 15B3


GRAM - 24 hour 15B3


GRAM - 48 hour 15B3

Cultural Characteristics of Bacterial Colonies

Culture No. 1583

I. Surface Colonies. Age 8 days, on TSA medium.

a. Microscopic appearance.

1. Size 2mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.

Topography- smooth, rough, wrinkled, contoured, straited, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. b½na (Flame)

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening. fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, filamentous, curled.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.,

d. Odor. Cooking Cauliflower

Name of organism Corynebacterium sp.* Studied by Dr. W.B.Bollen Culture No. 16

Source White Mountain Habitat Soil Date August 5, 1965

Descriptions (*Underscore required terms.*)	Sketches
CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 25 °C. Vegetative cells: Age: Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.* Motility in broth: – Flagella: – Size: 0.9-1.6 x 0.3-0.4 Irregular forms: – Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age: Endospores: Shape: *spherical, ellipsoid, cylindrical.* Position: *central to excentric, terminal, subterminal.*	SEE PAGE 27 FOR PHOTOMICROGRAPHS
STAINING CHARACTERISTICS Gram: + Age: 24hr. Method Kopeloff (modified) Special stains:	
AGAR STROKE Age: 24 hr. Temp. 25 °C. Amount of growth: *scanty, moderate, abundant.* Form: *filiform, echinulate, beaded, spreading, rhizoid.* Consistency: *butyrous, viscid, membranous, brittle.* Chromogenesis: ; *fluorescent, iridescent, photogenic.* 2fb Bamboo	
AGAR COLONIES Age: 8 day Temp. 25 °C. Form: *punctiform, circular, filamentous, rhizoid, irregular.* Elevation: *effuse, flat, raised, convex.* Surface: *smooth, contoured, radiate, concentric, rugose.* Margin: *entire, undulate, erose, filamentous, curled.* Density: *opaque, translucent.*	
NUTRIENT BROTH Age: 2 day Temp. 25 °C. Surface growth: *none, ring, pellicle, flocculent, membranous.* Subsurface growth: *none, turbid, granular.* Amount of growth: *scanty, moderate, abundant.* Sediment: *none, granular, flocculent, viscid, flaky.*	
GELATIN STAB Age: 5 day Temp. 25 °C. Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.* Rate: *slow, moderate, rapid.*	
OTHER MEDIA Age: Temp. °C. Potato slant: 1½ic Lt. Antique Gold Soybean Infusion agar: 3 ic Camel abundant Fat Agar: 2ca to 2ea Lt. Ivory to Lt. Wheat Glucose Nitrate agar: scant	

FERMENTATION Temp. 25 °C.

Medium: Nutrient Broth Carbohydrate: 1% Indicator: BCP	Glucose	Lactose	Sucrose	Xylose	
Acid in 10 days	+	=	+	+	
Acid in days					
Gas in 10 days	–	=	=	=	
Gas in days					

ACTION ON MILK Temp. 25 °C.

Indicator: Litmus	Days					
			13	14		
Reaction	neutral					
Acid curd						
Rennet curd						
Peptonization				+		
Reduction (before coagulation)			+			

* Soil diptheroid, no described species.

26

ACTION ON NITRATES

Medium 1% KNO_3 broth Temp. 25°C.

Nitrite: .....d. ; .....d. ; -3 d.

Gas (N): .....d. ; .....d. ; -3 d.

INDOLE PRODUCTION

Medium Tryptophane broth Age 10 day

Method: Kovac's Temp. 28°C.

Indole: *present, absent.*

HYDROGEN SULFIDE PRODUCTION

Medium Pb Acetate & Age 10 day

H_2S: *present, absent.* Thio-iron Temp. 28°C.

RELATION TO FREE OXYGEN Catalase: Positive

Medium Nutrient agar-Dextrose Age 10 day

Method: Shake Tubes Temp. 28°C.

TEMPERATURE RELATIONS

Growth in refrigerator (10°C.): *present, absent.*

Growth at room temperature (28°C.): *present, absent.*

Growth at 37° C.: *present, absent.*

Growth at 50° C.: *present, absent.*

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

Anaerobic growth: *present, absent.*

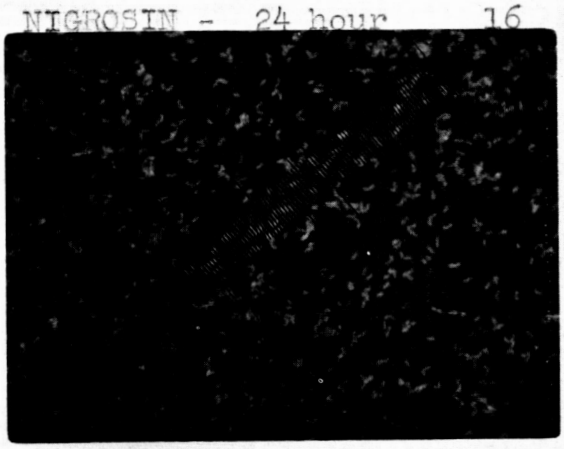
Pasteurization survival, 80°C. 10 minutes: Negative

ADDITIONAL TESTS

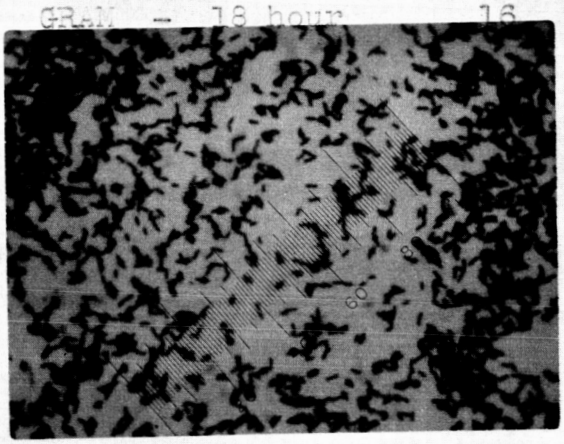
Casein Hydrolysis:	Positive
Fat Hydrolysis:	Negative
Gelatin Hydrolysis:	Positive
Starch Hydrolysis:	Negative
Urea Hydrolysis:	Negative
NH_4 from Peptone:	Positive
Metabolism:	Oxidizes Glucose, Xylose Nonox.-nonferm. Sucrose, Lactose
Acetyl methyl carbinol:	Negative
NH_4 as sole Nitrogen source:	Positive
Sole Carbon sources: Citrate -	Positive
Glucose -	Negative
Sucrose -	Positive - slight
Xylose -	Positive - slight
Methylene blue Reduction:	Positive
Salt tolerances: 2% -	Positive
7% -	Positive
10% -	Positive
Cellulose Digestion:	Negative
Selenite-nutrient agar:	Positive

1000x

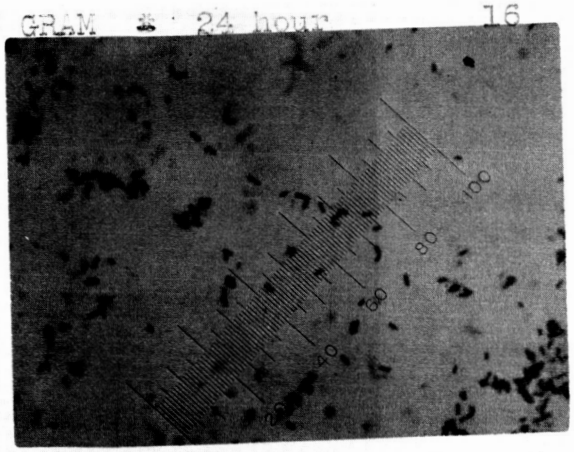
NIGROSIN - 24 hour 16



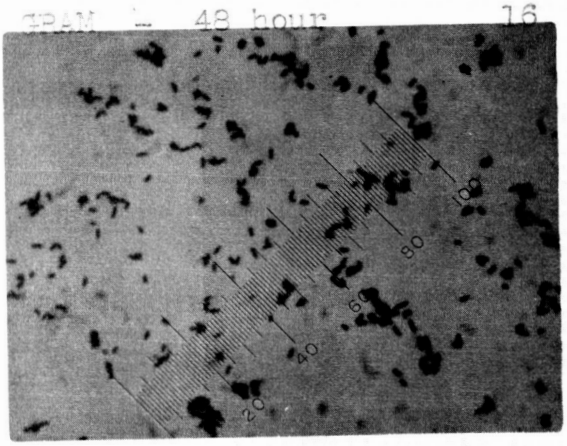
GRAM - 18 hour 16



GRAM - 24 hour 16



GRAM - 48 hour 16



Cultural Characteristics of Bacterial Colonies

Culture No. 16

I. Surface Colonies. Age 8 days, on TSA medium.

a. Microscopic appearance.

1. Size 3mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.

Topography- smooth, rough, wrinkled, contoured, straited, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 2fb Bamboo

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, filamentous, curled.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.,

d. Odor. Fecal

Name of organism Corynebacterium sp.* Studied by Dr. W.B.Bollen Culture No. 19E1

Source White Mountain Habitat Soil Date August 4, 1965

Descriptions (*Underscore required terms.*)	Sketches
CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 25 °C. Vegetative cells: Age: 24hr. Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.* Motility in broth: 0- Flagella: - Size: 3.00x.8µ Irregular forms: 1.5-4.5x0.75-1.0µ Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age: Endospores: Shape: *spherical, ellipsoid, cylindrical.* Position: *central to excentric, terminal, subterminal.*	SEE PAGE 31 FOR PHOTOMICROGRAPHS
STAINING CHARACTERISTICS Gram: + Age: 24hr. Method Kopeloff (modified) Special stains:	
AGAR STROKE Age: 24 hr. Temp. 25 °C. Amount of growth: *scanty, moderate, abundant.* Form: *filiform, echinulate, beaded, spreading, rhizoid.* Consistency: *butyrous, viscid, membranous, brittle.* Chromogenesis: ; *fluorescent, iridescent, photogenic.* 3ea Lt. Mellon Yellow	
AGAR COLONIES Age: 8 day Temp. 25 °C. Form: *punctiform, circular, filamentous, rhizoid, irregular.* Elevation: *effuse, flat, raised, convex.* Surface: *smooth, contoured, radiate, concentric, rugose.* Margin: *entire, undulate, erose, filamentous, curled.* Density: *opaque, translucent.*	
NUTRIENT BROTH Age: 2 day Temp. 25c. Surface growth: *none, ring, pellicle, flocculent, membranous.* Subsurface growth: *none, turbid, granular.* Amount of growth: *scanty, moderate, abundant.* Sediment: *none, granular, flocculent, viscid, flaky.*	
GELATIN STAB Age: 2 on Temp. 25 °C. Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.* Rate: *slow, moderate, rapid.*	
OTHER MEDIA Age: Temp. °C. Potato slant: 4gc Nude Tan ab. Soybean Infusion Agar: 3ea - 3ca Lt.MellonYellow to Pearl pink ab. Fat Agar: 3ea Lt. Mellon Yellow ab. Glucose Nitrate Agar: scant	

FERMENTATION Temp. 25 °C.	Glucose	Lactose	Sucrose	Xylose	
Medium: Nutrient broth Carbohydrate: 1 % Indicator: BCP					
Acid in 10 days	=	=	=	+	
Acid in days					
Gas in 10 days	=	=	=	-	
Gas in days					

ACTION ON MILK Temp. 25 °C.	Days						
Indicator: Litmus	4						
Reaction	alkaline						
Acid curd							
Rennet curd							
Peptonization							
Reduction (before coagulation)							

* Soil diptheroid; does not fit any described species.

ACTION ON NITRATES
Medium: 1 % KNO_3 broth Temp 28 °C.
Nitrite: .....d. ; .....d. ; -3 d.
Gas (N): .....d. ; .....d. ; -3 d.

INDOLE PRODUCTION
Medium: Tryptophane broth Age: 10 day
Method: Kovac's Temp 28 °C.
Indole: *present, absent.*

HYDROGEN SULFIDE PRODUCTION
Medium: Pb Acetate & Age: 10 day
H_2S: *present, absent.* Thio-iron Temp 28 °C.

TEMPERATURE RELATIONS
Growth in refrigerator (10°C.): *present, absent.*
Growth at room temperature (28°C.): *present, absent.*
Growth at 37° C.: *present, absent.*
Growth at 50° C.: *present, absent.*

RELATION TO FREE OXYGEN Catalase: Positive
Medium Dextrose-nutrient agar Age: 10day
Method: Shake Tubes Temp 28 °C.

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

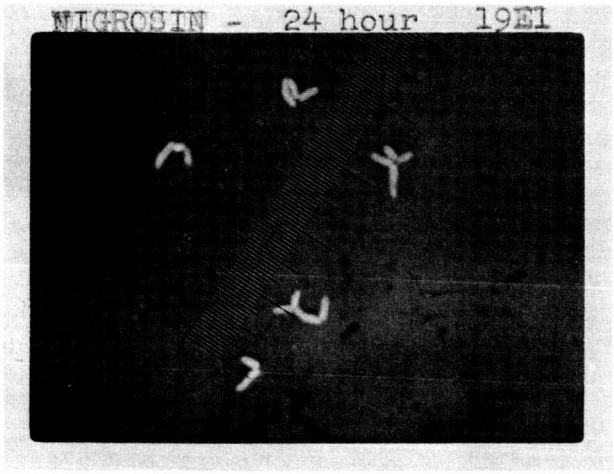
Anaerobic growth: *present, absent.*

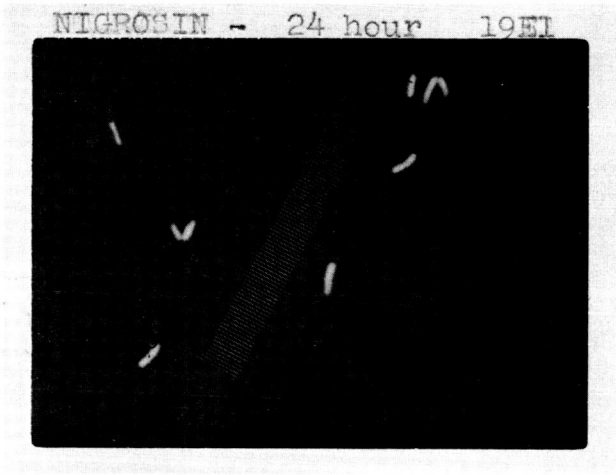
Pasteurization survival, 80°C. 10 minutes: Negative

ADDITIONAL TESTS

Casein Hydrolysis:		Negative
Fat Hydrolysis:		Negative
Gelatin Hydrolysis:		Negative
Starch Hydrolysis:		Negative
Urea Hydrolysis:		Positive
NH_4 from Peptone:		Positive
Metabolism:		Ferments Glucose, Sucrose Nonox-nonferm. Lactose, Xylose
Acetyl methyl carbinol:		Negative
NH_4 as sole Nitrogen source:		Positive
Sole Carbon sources:	Citrate -	Positive
	Glucose -	Positive
	Sucrose -	Positive
	Xylose -	Positive - slight
Methylene blue Reduction:		Negative
Salt tolerances:	2% -	Positive
	7% -	Positive
	10% -	Positive
Cellulose Digestion:		Negative
Selenite-nutrient agar:		Positive - slight

1000x


NIGROSIN - 24 hour 19E1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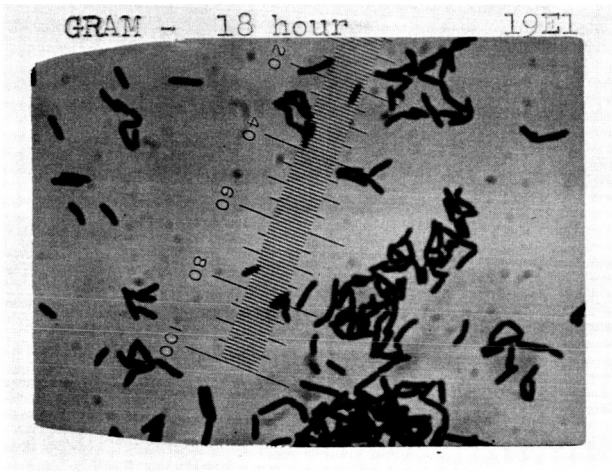
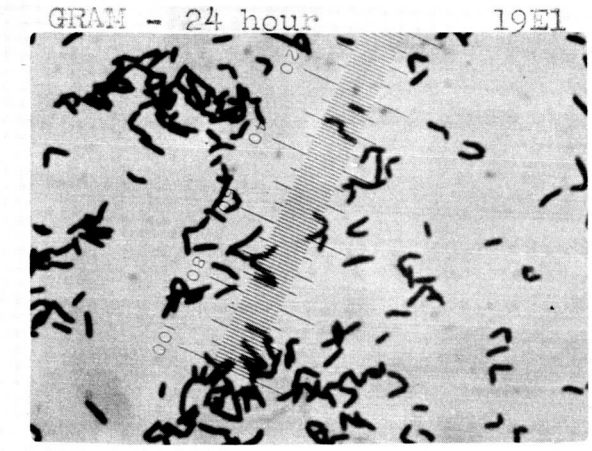
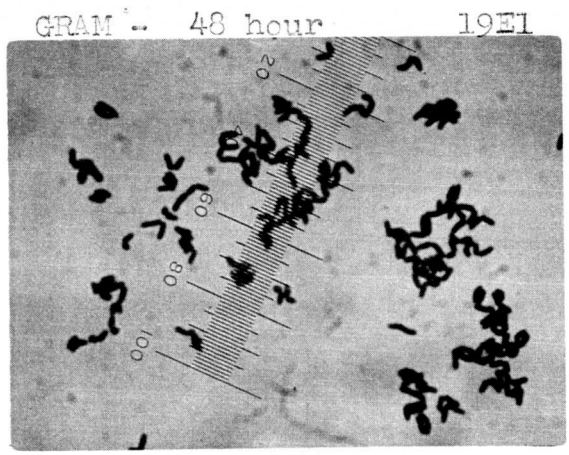

GRAM - 18 hour 19E1


GRAM - 24 hour 19E1


GRAM - 48 hour 19E1

Cultural Characteristics of Bacterial Colonies

Culture No. 1921

I. Surface Colonies. Age 8 days, on TSA medium.

a. Microscopic appearance.

1. Size, 1 mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.

Topography- smooth, rough, wrinkled, contoured, straited, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 3ea (Lt. Mellow Yellow)

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, filamentous, curled.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.,

d. Odor. Fecal

Name of organism Corynebacterium sp.* Studied by Dr. W.B.Bollen Culture No. 19G1

Source White Mountain Habitat Soil Date August 5, 1965

Descriptions (*Underscore required terms.*) | Sketches

CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 25 °C.

Vegetative cells: Age: 24 hr.

Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filamentis.*

Motility in broth: – Flagella: –

Size: 3.58 x 0.92μ
2.25-6.0 x 0.75-1.25μ

Irregular forms:

Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age:

Endospores:

Shape: *spherical, ellipsoid, cylindrical.*

Position: *central to excentric, terminal, subterminal.*

SEE PAGE 35 FOR PHOTOMICROGRAPHS

STAINING CHARACTERISTICS

Gram: + Age: 24 hr Method: Kopeloff's (modified)

Special stains:

AGAR STROKE Age: 24 hr. Temp. 25 °C.

Amount of growth: *scanty, moderate, abundant.*

Form: *filiform, echinulate, beaded, spreading, rhizoid.*

Consistency: *butyrous, viscid, membranous, brittle.*

Chromogenesis: ; *fluorescent, iridescent, photogenic.*

4ea Lt. Apricot

AGAR COLONIES Age: 7 day Temp. 25°C.

Form: *punctiform, circular, filamentous, rhizoid, irregular.*

Elevation: *effuse, flat, raised, convex.* papillate

Surface: *smooth, contoured, radiate, concentric, rugose.*

Margin: *entire, undulate, erose, filamentous, curled.*

Density: *opaque, translucent.*

NUTRIENT BROTH Age: 2 day Temp. 25°C.

Surface growth: *none, ring, pellicle, flocculent, membranous.*

Subsurface growth: *none, turbid, granular.*

Amount of growth: *scanty, moderate, abundant.*

Sediment: *none, granular, flocculent, viscid, flaky.*

1200

GELATIN STAB Age: 30 day Temp. 25 °C.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *slow, moderate, rapid.*

OTHER MEDIA Age: Temp. °C.

Potato slant:	4gc	Nude Tan	abundant
Soybean Infusion agar:	4ea	Flesh Pink	
Glucose nitrate agar:		White	abundant
Fat agar:	3ca	Pearl pink	

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth Carbohydrate: 1% Indicator: BCP

	Glucose	Lactose	Sucrose	Xylose	
Acid in 10 days	–	+	+	+	
Acid in days					
Gas in 10 days	+	+	+	+	
Gas in days					

ACTION ON MILK Temp. 25 °C.

Indicator:	Days					
Litmus	2					
Reaction	ALKALINE					
Acid curd						
Rennet curd						
Peptonization						
Reduction (before coagulation)						

* Soil diptheroid; no described species.

34

ACTION ON NITRATES
Medium: 1% KNO_3 broth Temp. 28°C.
Nitrite: + 3 d. ; ..... d. ; ..... d.
Gas (N): - 3 d. ; ..... d. ; ..... d.

INDOLE PRODUCTION
Medium: Tryptophane broth Age: 10 day
Method: Kovac's Temp. 28 °C.
Indole: *present, absent*

HYDROGEN SULFIDE PRODUCTION
Medium: Pb Acetate & Age: 10 day
H_2S: *present, absent.* Thio-iron Temp. 28°C.

RELATION TO FREE OXYGEN Catalase: Positive
Medium: Dextrose-nutrient agar Age: 10 day
Method: Shake tubes Temp. 28 °C.

TEMPERATURE RELATIONS
Growth in refrigerator (10 C.): *present, absent.*
Growth at room temperature (28 C.): *present, absent.*
Growth at 37° C.: *present, absent.*
Growth at 50° C.: *present, absent.*

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

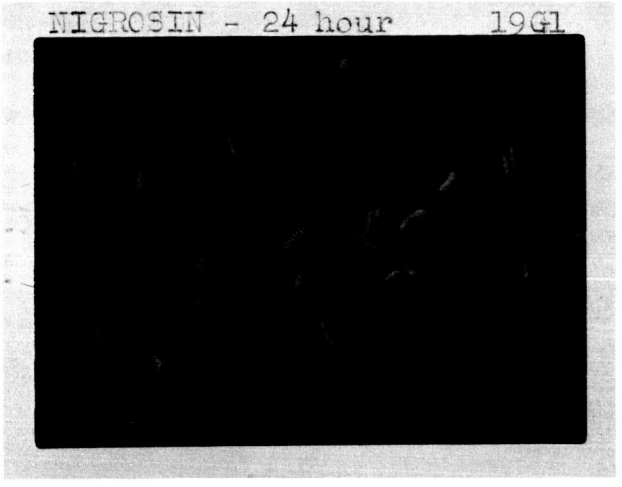
Anaerobic growth: *present, absent.*

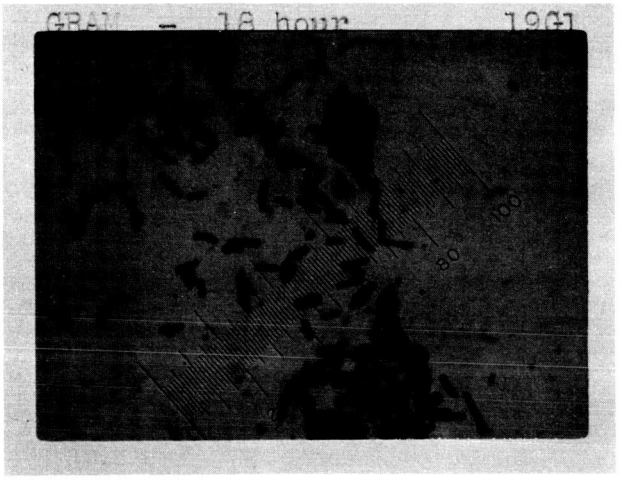
Pasteurization survival, 80°C. 10 minutes: Negative

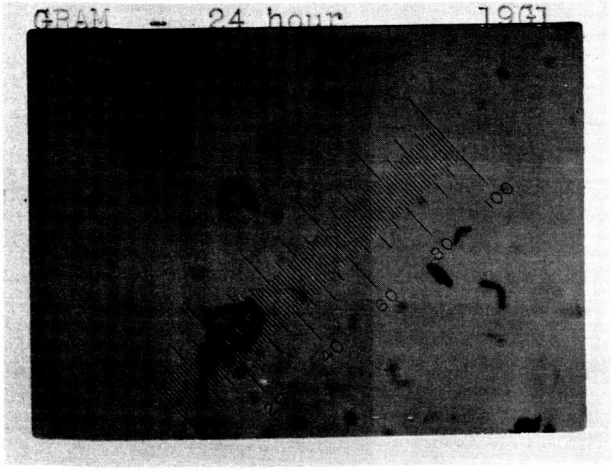
ADDITIONAL TESTS

Casein Hydrolysis:	Negative
Fat Hydrolysis:	Negative
Gelatin Hydrolysis:	Negative
Starch Hydrolysis:	Negative
Urea Hydrolysis:	Positive
NH_4 from Peptone:	Positive
Metabolism:	Oxidizes Glucose, Sucrose Nonox.-nonferm. Lactose, Xylose
Acetyl methyl carbinol:	Negative
NH_4 as sole Nitrogen source:	Positive
Sole Carbon sources: Citrate -	Positive
Glucose -	Positive
Sucrose -	Positive
Xylose -	Positive
Methylene blue reduction:	Positive
Salt tolerances: 2% -	Positive
7% -	Positive
10% -	Positive
Cellulose Digestion:	Negative
Selenite-nutrient agar:	Positive

1000x


NIGROSIN - 24 hour 19G1


GRAM - 18 hour 19G1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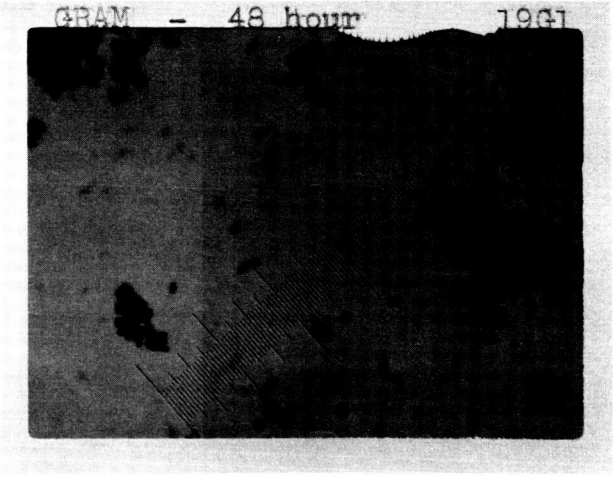

GRAM - 48 hour 19G1

Cultural Characteristics of Bacterial Colonies

Culture No. 1961

I. Surface Colonies. Age 8 days, on TSA medium.

a. Microscopic appearance.

1. Size, 2mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.

Topography- smooth, rough, wrinkled, contoured, straited, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 4ea Lt. Apricot

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, filamentous, curled.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.,

d. Odor. Fecal

Name of organism Corynebacterium sp.* Studied by Dr. W. B. Bollen Culture No. 19X

Source White Mountain Habitat Soil Date August 5, 1965

Descriptions (*Underscore required terms.*)	Sketches
CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 25 °C. Vegetative cells: Age: Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.* Motility in broth: - Flagella: - Size: 2.5 x 0.8 µ Irregular forms: 2.0-4.0 x 0.5 µ Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age: Endospores: Shape: *spherical, ellipsoid, cylindrical.* Position: *central to excentric, terminal, subterminal.*	SEE PAGE 39 FOR PHOTOMICROGRAPHS
STAINING CHARACTERISTICS Gram: + Age: 24hr. Method: Kopeloff (modified) Special stains:	
AGAR STROKE Age: 24hr. Temp. 25 °C. Amount of growth: *scanty, moderate, abundant.* Form: *filiform, echinulate, beaded, spreading, rhizoid.* Consistency: *butyrous, viscid, membranous, brittle.* Chromogenesis: *; fluorescent, iridescent, photogenic.* 3ea Lt. Mellon Yellow	
AGAR COLONIES Age: 8 day Temp. 25 °C. Form: *punctiform, circular, filamentous, rhizoid, irregular.* Elevation: *effuse, flat, raised, convex.* umbonate Surface: *smooth, contoured, radiate, concentric, rugose.* rough Margin: *entire, undulate, erose, filamentous, curled.* crenate Density: *opaque, translucent.*	
NUTRIENT BROTH Age: 2 day Temp. 25c. Surface growth: *none, ring, pellicle, flocculent, membranous.* Subsurface growth: *none, turbid, granular.* Amount of growth: *scanty, moderate, abundant.* Sediment: *none, granular, flocculent, viscid, flaky.*	
GELATIN STAB Age: 12 day Temp. 25 °C. Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.* Rate: *slow, moderate, rapid.*	

OTHER MEDIA Age: Temp. °C.

Potato slant:	4gc	Nude Tan	Abundant
Fat agar:	3ea	Lt. Mellon Yellow	Abundant
Soybean Infusion agar:	3ca	Pearl Pink	Abundant
Glucose nitrate agar:			Scant

FERMENTATION Temp. 25 °C.

Medium: Nutrient broth 1% Carbohydrate: Indicator: BCP	Glucose	Lactose	Sucrose	Xylose	
Acid in 2 days	=	=	=	=	
Acid in 5 days	-	-	+	-	
Gas in 2 days	-	-	-	-	
Gas in 5 days	-	-	-	-	

ACTION ON MILK Temp. 25 °C.

Indicator:	Days						
Litmus	2						
Reaction	ALKALINE.						
Acid curd							
Rennet curd							
Peptonization							
Reduction (before coagulation)							

* Soil diptheroid.

ACTION ON NITRATES
Medium: 1% KNO_3 broth Temp 28 °C.
Nitrite: .....d. ; .....d. ; +3 d.
Gas (N): .....d. ; .....d. ; −3 d.

INDOLE PRODUCTION
Medium: Tryptophane broth Age: 10 day
Method: Kovac's Temp. 28 °C.
Indole: *present, absent.*

HYDROGEN SULFIDE PRODUCTION
Medium: Pb acetate & Thio-iron Age: 10day Temp 28 °C.
H_2S: *present, absent.*

RELATION TO FREE OXYGEN Gelatin: Positive
Medium: Dextrose-Nutrient agar Age 10 day
Method: Shake Tubes Temp. 28c.

TEMPERATURE RELATIONS
Growth in refrigerator (10°C.): *present, absent.*
Growth at room temperature (28°C.): *present, absent.*
Growth at 37° C.: *present, absent.*
Growth at 50° C.: *present, absent.*

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

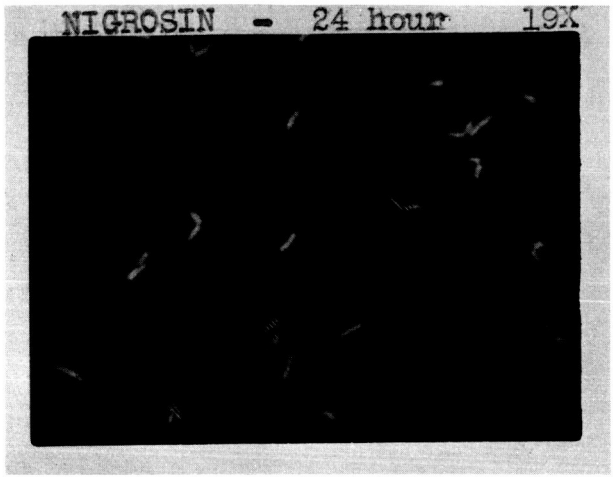
Anaerobic growth: *present, absent.*

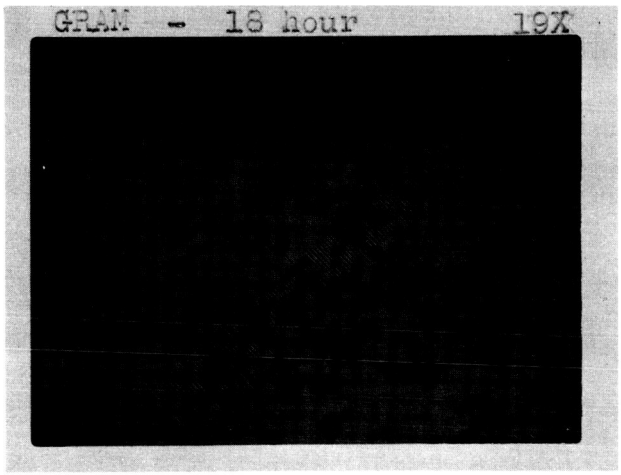
Pasteurization survival, 80°C. 10 minutes: Negative

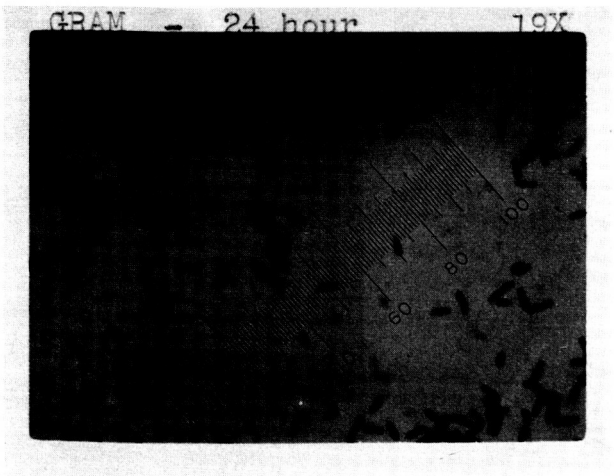
ADDITIONAL TESTS

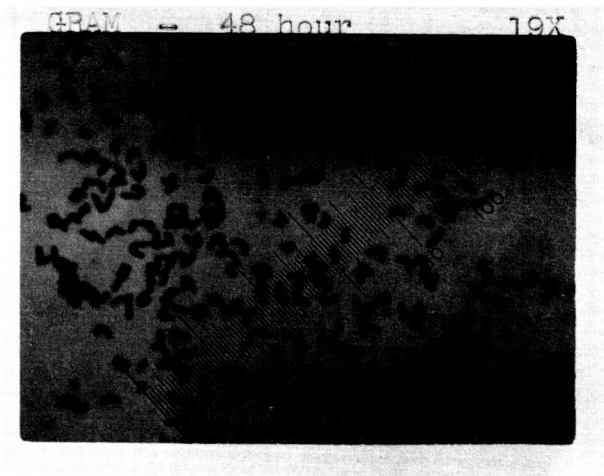
Casein Hydrolysis:	Negative
Fat Hydrolysis:	Negative
Gelatin Hydrolysis:	Negative
Starch Hydrolysis:	Negative
Urea Hydrolysis:	Positive
NH_4 from Peptone:	Positive
Metabolism:	Oxidizes Glucose, Sucrose Nonox.-nonferm. Lactose, Xylos
Acetyl methyl carbinol:	Negative
NH_4 as sole Nitrogen source:	Positive
Sole Carbon sources: Citrate -	Positive
Glucose -	Positive
Sucrose -	Positive - slight
Xylose -	Positive -, slight
Methylene blue Reduction:	Positive - slow
Salt tolerances: 2% -	Positive
7% -	Positive - slight
10% -	Positive - slight
Cellulose Digestion:	Negative
Selenite nutrient agar:	Positive

1000x


NIGROSIN - 24 hour 19X


GRAM - 18 hour 19X


GRAM - 24 hour 19X


GRAM - 48 hour 19X

Cultural Characteristics of Bacterial Colonies

Culture No. 19X

I. Surface Colonies. Age 5 days, on TSA medium.

a. Microscopic appearance.

1. Size, 2mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.

Topography- smooth, rough, wrinkled, contoured, straited, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 3ea (lt. Mellow Yellow)

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, filamentous, curled.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.,

d. Odor. cooking cauliflower

Name of organism Corynebacterium sp.* Studied by Dr. W.B.Bollen Culture No. 19Y

Source White Mountain Habitat Soil Date August 6, 1965

Descriptions (*Underscore required terms.*)	Sketches
CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 25 °C. Vegetative cells: Age: 18 hr. Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.* Motility in broth: + Flagella: Peritrichous Size: 2.90 x 0.85 - 1.0 x 0.5-0.75 Irregular forms: coccoid Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age: Endospores: Shape: *spherical, ellipsoid, cylindrical.* Position: *central to excentric, terminal, subterminal.*	SEE PAGE 43 FOR PHOTOMICROGRAPHS
STAINING CHARACTERISTICS Gram: + Age: 24hr. Method Kopeloff (modified) Special stains:	
AGAR STROKE Age: 24 hr. Temp. 25 °C. Amount of growth: *scanty, moderate, abundant.* Form: *filiform, echinulate, beaded, spreading, rhizoid.* Consistency: *butyrous, viscid, membranous, brittle.* Chromogenesis: ; *fluorescent, iridescent, photogenic.* 3ca Pearl Pink	
AGAR COLONIES Age: 24 hour Temp. 25 °C. Form: *punctiform, circular, filamentous, rhizoid, irregular.* Elevation: *effuse, flat, raised, convex.* umbonate Surface: *smooth, contoured, radiate, concentric, rugose.* Margin: *entire, undulate, erose, filamentous, curled.* crenate Density: *opaque, translucent.*	
NUTRIENT BROTH Age: 2 day Temp. 25 °C. Surface growth: *none, ring, pellicle, flocculent, membranous.* Subsurface growth: *none, turbid, granular.* Amount of growth: *scanty, moderate, abundant.* Sediment: *none, granular, flocculent, viscid, flaky.*	
GELATIN STAB Age: 30 day Temp. 25c. Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.* Rate: *slow, moderate, rapid.*	
OTHER MEDIA Age: Temp. °C.	
Potato slant: 4ga-4gc Apricot-Nude Tan Soybean Infusion agar: White Abundant Fat agar: 3ca Pearl Pink Abundant Glucose nitrate agar: Scant	

FERMENTATION Temp. 25 °C.					
Medium: Nutrient broth Carbohydrate: 1% Indicator: BCP	Glucose	Lactose	Sucrose	Lactose	
Acid in 10 days	=	=	=	+	
Acid in days					
Gas in 10 days	=	=	=	=	
Gas in days					

ACTION ON MILK Temp. 25 °C.						
Indicator: Litmus	Days					
	2					
Reaction	=		NEUTRAL			
Acid curd						
Rennet curd						
Peptonization						
Reduction (before coagulation)	+					

gas

* Soil diptheroid.

42

ACTION ON NITRATES

Medium: 1% KNO_3 broth Temp 28 °C.

Nitrite: .....d. ; .....d. ; -3 d.

Gas (N): .....d. ; .....d. ; -3 d.

INDOLE PRODUCTION

Medium: Tryptophane broth Age: 10 day

Method: Kovac's Temp. 28°C.

Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION

Medium: Pb Acetate & Thio-iron Age: 10 day

H_2S: *present*, *absent*. Temp 28 °C.

TEMPERATURE RELATIONS

Growth in refrigerator (10°C.): *present*, *absent*.

Growth at room temperature (28°C.): *present*, *absent*.

Growth at 37° C.: *present*, *absent*.

Growth at 50° C.: *present*, *absent*.

RELATION TO FREE OXYGEN Catalase: Positive

Medium: Dextrose-Nutrient agar 10 day

Method: Shake tubes Temp. 28°C.

Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.

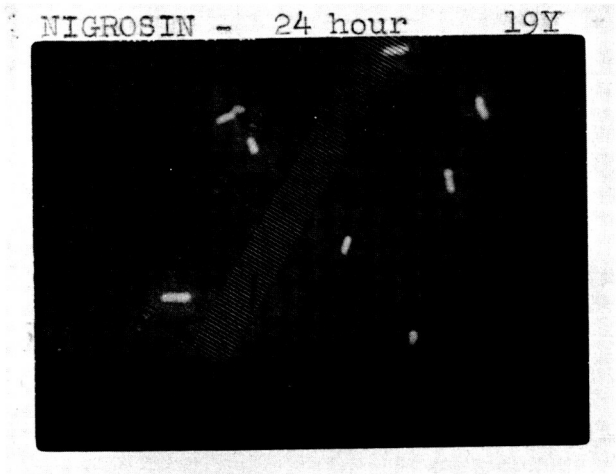
Anaerobic growth: *present*, *absent*.

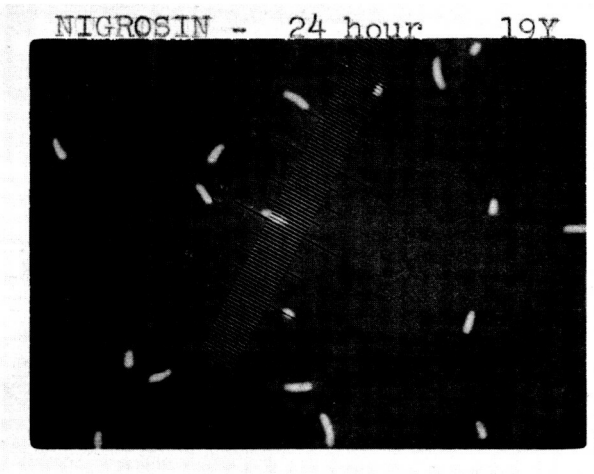
Pasteurization survival, 80°C. 10 minutes: Negative

ADDITIONAL TESTS

Casein Hydrolysis:	Negative
Fat Hydrolysis:	Negative
Gelatin Hydrolysis:	Negative
Starch Hydrolysis:	Negative
Urea Hydrolysis:	Positive
NH_4 from Peptone:	Positive
Metabolism:	Ferments Glucose, Sucrose Nonox.-nonferm. Lactose, Xylo
Acetyl methyl carbinol:	Negative
NH_4 as sole Nitrogen source:	Positive
Sole Carbon sources: Citrate -	Positive
Glucose -	Positive
Sucrose -	Positive
Xylose -	Positive - slight
Methylene blue Reduction:	Positive
Salt tolerances: 2% -	Positive
7% -	Negative
10% -	Negative
Cellulose Digestion:	Negative
Selenite-nutrient agar:	Positive

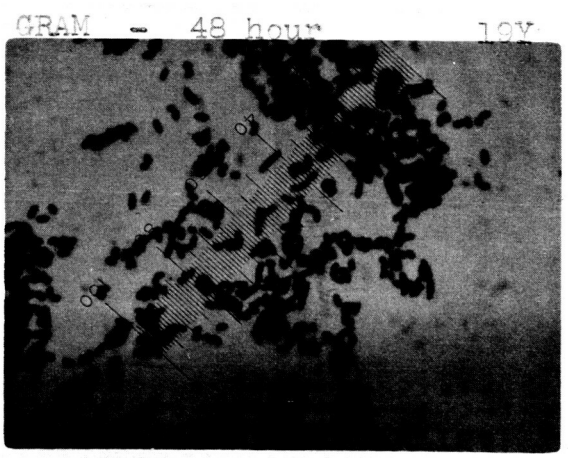
1000x


NIGROSIN - 24 hour 19Y


NIGROSIN - 24 hour 19Y


GRAM - 18 hour 19Y


GRAM - 24 hour 19Y


GRAM - 48 hour 19Y

Cultural Characteristics of Bacterial Colonies

Culture No. 194

I. Surface Colonies. Age 8 days, on TSA medium.

a. Microscopic appearance.

1. Size 2mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.

Topography- smooth, rough, wrinkled, contoured, straited, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 3ca Pearl Pink

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, filamentous, curled.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.,

d. Odor. cooking cabbage

Name of organism Corynebacterium sp.* Studied by Dr. W.B.Bollen Culture No. 122B

Source Habitat Soil Date August 6, 1965

Descriptions (*Underscore required terms.*)	Sketches
CELL MORPHOLOGY Medium: Trypticase soy agar Temp. 25 °C. Vegetative cells: Age: 24 hr. Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.* Motility in broth: — Flagella: — Size: 2.50 x 0.88 1.5-4.0 x 0.75-1.0µ Irregular forms: Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age: Endospores: Shape: *spherical, ellipsoid, cylindrical.* Position: *central to eccentric, terminal, subterminal.*	SEE PAGE 47 FOR PHOTOMICROGRAPHS
STAINING CHARACTERISTICS Gram: + Age: 24hr. Method: Kopeloff (modified) Special stains:	
AGAR STROKE Age 24 hr. Temp. 25 °C. Amount of growth: *scanty, moderate, abundant.* Form: *filiform, echinulate, beaded, spreading, rhizoid.* Consistency: *butyrous, viscid, membranous, brittle.* Chromogenesis: ; *fluorescent, iridescent, photogenic.* 3ea Lt. Mellon Yellow	
AGAR COLONIES Age: 8 day Temp. 25 °C. Form: *punctiform, circular, filamentous, rhizoid, irregular.* Elevation: *effuse, flat, raised, convex.* Surface: *smooth, contoured, radiate, concentric, rugose.* Margin: *entire, undulate, erose, filamentous, curled.* Density: *opaque, translucent.*	
NUTRIENT BROTH Age: 1 - 8 da. Temp. 25 °C. Surface growth: *none, ring, pellicle, flocculent, membranous.* Subsurface growth: *none, turbid, granular.* Amount of growth: *scanty, moderate, abundant.* Sediment: *none, granular, flocculent, viscid, flaky.*	
GELATIN STAB Age: 12 day + Temp. 25 °C. Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.* Rate: *slow, moderate, rapid.*	
OTHER MEDIA Age: Temp. °C.	
Potato slant: 4gc Nude Tan Abundant Soybean Infusion agar: 3oa Pearl Pink Abundant Glucose nitrate agar: White Abundant Fat agar: 3oa Pearl Pink Abundant	

FERMENTATION Temp. 25 °C.					
Medium: Nutrient broth Carbohydrate: 1% Indicator: BCP	Glucose	Lactose	Sucrose	Xylose	
Acid in 10 days	=	=	=	=	
Acid in days					
Gas in 10 days	=	=	=	=	
Gas in days					

ACTION ON MILK Temp. 25 °C.	Days					
Indicator: Litmus	2					
Reaction	alkaline					
Acid curd						
Rennet curd						
Peptonization						
Reduction (before coagulation)						

* Soil diptheroid.

46

ACTION ON NITRATES
Medium: 1% KNO3 broth Temp. 28°C.
Nitrite: .....d. ; .....d. ; -3.....d.
Gas (N): .....d. ; .....d. ; -3.....d.

INDOLE PRODUCTION
Medium: Tryptophane broth Age: 10 day
Method: Kovac's Temp. 28°C.
Indole: *present*, *absent*.

HYDROGEN SULFIDE PRODUCTION
Medium: Pb Acetate & Age: 10 day
H_2S: *present*, *absent*. Thio-Iron Temp. 28°C.

RELATION TO FREE OXYGEN Catalase: Positive
Medium: Dextrose-nutrient agar Age: 10 day
Method: Shake Tubes Temp. 28°C.
Aerobic growth: *absent*, *present*, *better than anaerobic growth*, *poorer than anaerobic growth*.
Anaerobic growth: *present*, *absent*.

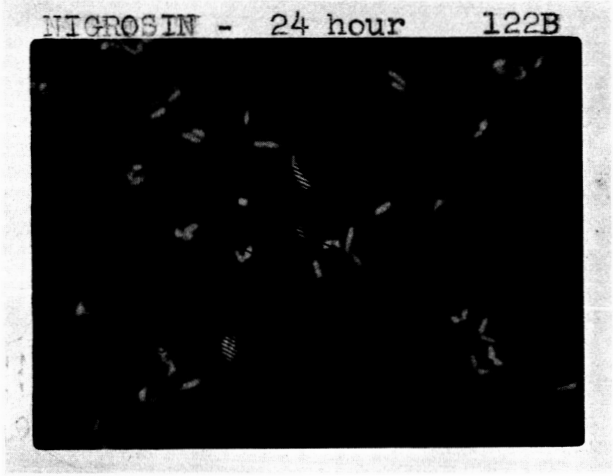
TEMPERATURE RELATIONS
Growth in refrigerator (10 °C.): *present*, *absent*.
Growth at room temperature (28C.): *present*, *absent*.
Growth at 37° C.: *present*, *absent*.
Growth at 50° C.: *present*, *absent*.

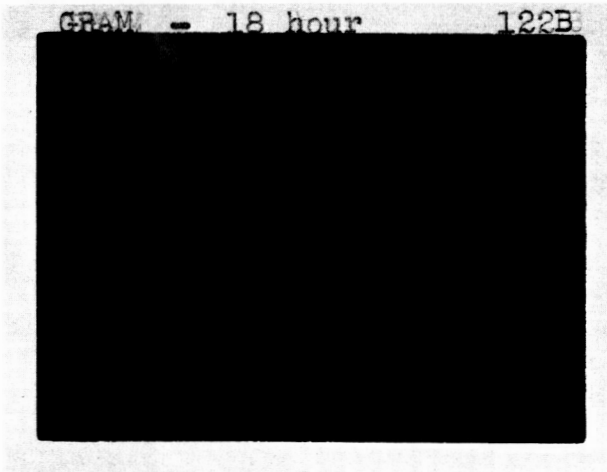
Pasteurization survival, 80°C. 10 minutes: Negative

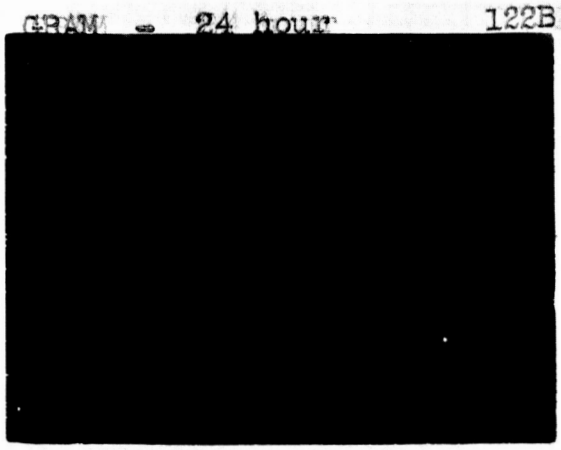
ADDITIONAL TESTS

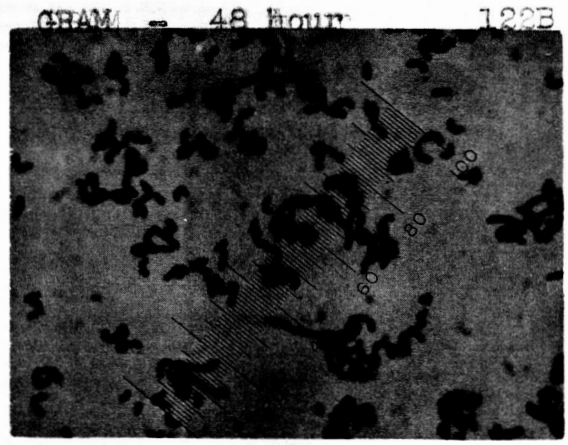
Casein Hydrolysis:	Negative
Fat Hydrolysis:	Negative
Gelatin Hydrolysis:	Negative
Starch Hydrolysis:	Negative
Urea Hydrolysis:	Positive
NH_4 from Peptone:	Positive
Metabolism:	Nonox.-nonferm.
Acetyl methyl carbinol:	Negative
NH_4 as sole Nitrogen source:	Positive
Sole Carbon sources: Citrate -	Positive
Glucose -	Positive
Sucrose -	Positive
Xylose -	Positive
Methylene blue reduction:	Positive
Salt tolerances: 2% -	Positive
7% -	Positive
10% -	Positive
Cellulose Digestion:	Negative
Selenite-nutrient agar:	Positive

1000x


NIGROSIN - 24 hour 122B


GRAM - 18 hour 122B


GRAM - 24 hour 122B


GRAM - 48 hour 122B

Cultural Characteristics of Bacterial Colonies

Culture No. 122 B

I. Surface Colonies. Age 8 days, on tsa medium.

a. Microscopic appearance.

1. Size, 3mm

2. Shape: Outline- punctiform, circular, oval, irregular, filamentous, rhizoid.

Elevation- effuse, flat, raised, convex, rugose, papillate, umbonate, pulvinate.

Topography- smooth, rough, wrinkled, contoured, straited, concentrically ringed, radially ridged.

Habit- compact, spreading.

3. Optical properties:

(a) Color: Color Harmony Manual No. 3ea lt. Mellow Yellow.

(b) Appearance by reflected light- dull, opalescent, iridescent, glistening, fluorescent.

(c) Appearance by transmitted light- transparent, translucent, opaque.

b. Microscopic appearance (X100).

1. Margin- entire, granular, cleft, lobed, undulate, crenate, erose, ciliate, filamentous, curled.

2. Internal structure- amorphous, dense, granular (fine, coarse), filamentous, striated, interlaced.

c. Consistency- moist, slimy, soft, butyrous, waxy, tough, adherent, brittle.,

d. Odor. cooking cabbage